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Erratum

Vol. xlviii, page 401. Legend of figure 2, line 2, *after* "blood" *insert* 'and this at 29 by indifferent blood."

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THE HYPERGLYCEMIA-PROVOKING ABILITY OF ASPHYXIAL BLOOD

K. YAMAKAMI

From Tohoku Imperial University, Japan

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The rôle of adrenalin in the asphyxial hyperglycemia and glycosuria has been a question for a long time and is not yet decided.

Pollak (1), in classifying the various experimental conditions in which hyperglycemia occurs, regarded asphyxial hyperglycemia as due to a stimulation of the sympathetic nervous system akin to that produced by the injection of adrenalin. The experimental basis for this opinion lay in the fact that the asphyxial glycosuria and hyperglycemia could not be prevented by resecting the splanchinic nerves of both sides, though they were remarkably lowered in degree by this operation.

Emil Starkenstein (2) carried out a number of experiments in which he proved histologically a diminution of the chromaffin substance in the medullary cells of the adrenals of asphyxiated rabbits. Extracts from the adrenals of these asphyxiated rabbits also exhibited a lessened power to raise the blood pressure when injected into normal rabbits. The histological staining abnormities as well as the decrease of the blood-pressure-raising substance could be prevented by cutting the splanchinic nerves before suffocation. He presumed upon this experimental ground that the asphyxial stimulation acts first upon the blood-sugar-controlling centre of the brain as in the case of Piqûre, and then this stimulation is transmitted along the sympathetic nerve to the suprerenal, causing a hypersecretion of suprarenin by exciting the medullary cells and this adrenalin, transferred into the circulation, gives rise to the increased sugar content of the blood.

Czubalski (3), supporting the view that hypersecretion of adrenalin occurs in asphyxia, proved that the rise of the blood pressure of animals does not occur if previous to suffocation the adrenals are removed. Borberg, Frederica and many others carried out similar experiments to prove the rôle of the suprarenals or suprarenin in asphyxial glycosuria and hyperglycemia. But most of the methods of investigation employed by these authors were indirect and did not permit a definite decision as to the rôle of suprarenin.

In order to solve the question whether the adrenals are involved in asphyxial hyperglycemia, it seems to be the wisest method to study this hyperglycemia in animals whose adrenals were removed entirely. This is, however, not an easy task for most animals live only a short time after the operation. It would not be safe, as Stewart has pointed out, to take the experimental results obtained from animals in such a condition that they are about to die, as evidence of an important rôle of adrenalin in experimental diabetes. The only animal which can survive double adrenalectomy for long without any pathological symptoms, is the rabbit. Consequently most of the experiments in this field have been performed upon this animal. Many investigators have stated that hyperglycemia or glycosuria do not occur after removal of the adrenals.

The non-occurrence of Piqûre glycosuria after adrenalectomy was proved by Meyer (4), that of the glycosuria provoked by splanchnic stimulation was proved by Thomas (5) and Macleod (6), that of the CO-glycosuria was proved by Starkenstein. Kahn, Starkenstein and others are said to have proved that various kinds of glycosuria do not manifest themselves in rabbits even one year after adrenalectomy (7). Macleod and Pearce (8) obtained similar results in regard to hyperglycemia.

Kahn has reported in his more recent paper the non-occurrence of hyperglycemia following diabetic puncture, CO, diuretin, emotion and salt, in rabbits after successful adrenalectomy (9).

These results obtained by various authors might be considered as sufficient evidence to prove the indispensable intervention of the adrenals or of adrenalin in all experimental diabetes and hyperglycemias, except the renal and pancreas diabetes, if other authors had not obtained the opposite results. Unfortunately many exceptional cases have been observed, in which adrenalectomy of both sides did not prevent the experimental glycosuria or hyperglycemia, especially in other animals than rabbits. For instance, Wertheimer and Battez (10)

could obtain glycosuria in cats by Piqûre after adrenalectomy. Starkenstein observed a remarkable glycosuria produced by stimulating the splanchnic nerves for a long time in rabbits deprived of their adrenals.

Kahn comments upon the occasional occurrences of the glycosuria or hyperglycemia after adrenalectomy in the following words:

> Es wird also anzunehmen sein dass die auf dem direkten Wege erzeugte Reizung der sympatischen Nervenende in der Leber, weder vei der CO noch bei Piqûre hoch gradig genug sei, um zur plötzlichen ueberstürtzten Glykogen Mobilisierung zu rühren. Diese notwendige Erregungsgrösse wird erst durch die Addition von Adrenalin zu Wege gebracht. Wenn aber durch irgend eine unbekannte Ursache, die Erregungsgrösse erbracht im direkten Wege gross genug sei, dann treten die Glykosurie oder Hyperglykaemie in nebennierenlosen oder Splanchinicus resezierten Tieren auf.

There are too many presumptions which have not sufficient experimental foundation in the explanation offered by Kahn. But, in any case, this much seems to be true, that various experimental hyperglycemias can be made difficult to manifest themselves by extirpation of adrenals even in the presence of a sufficient deposit of liver glycogen. Besides there is one more possibility which may account for the hyperglycemia after extirpation of the suprarenals, in favor of the adrenalin theory, and that is the probability of adrenalin secretion from other glands than the adrenals. The carotid glands, Zuckerkandls glands, Luschkas glands and others which contain chromaffin cells may produce and secrete suprarenin. The amount of suprarenin produced from this source may be very small under normal conditions of life. But it is quite conceivable that this amount may rise compensatorily when the main sources of adrenalin are removed and extraordinary stimulations are applied to the secretory nerves.

Recent attempts to elucidate this problem have been made by two American investigators and their students, namely, Cannon and Stewart. They tried to determine the increase of adrenalin in the blood of animals under experimental conditions directly by means of segments of uterus and intestine. And curious to say, their results were just opposite to each other, Cannon and his students having obtained positive (11), Stewart and his collaborators having obtained negative results (12).

Stewart worked on cats in which one adrenal was removed, and the nerve supply for the other was cut off. It is not surprising that he was able to provoke asphyxial or other experimental hyperglycemia in such animals because, as was cited before, the experimental hyper-

glycemia manifests itself sometimes even in cats deprived of both adrenals. But his negative results, in attempting to detect the epinephrin output from the remaining adrenal, upon which experimental basis he infers that the asphyxial hyperglycemia occurs without any intervention of adrenalin, may have been due to the exhaustion of epinephrin, because the methods employed by Stewart to obtain the blood sample included numerous procedures which have been shown by various investigators to cause experimental hyperglycemia. The fixation of animals upon the operation table, narcotization, laparotomy, ligation of a large artery and vein, etc., have all been reported as causing a rise in blood sugar. And all of these hyperglycemias may be accompanied by hypersecretion of adrenalin, as many authors believe. Even if the asphyxial stimulation leads to hypersecretion from the adrenal glands, this secretory stimulation can not be very strong. The various manipulations involved in obtaining the asphyxial blood sample may very probably, therefore, have exhausted the adrenals to such a degree that no more epinephrin could be elicited from the glands by such a weak stimulation as asphyxia. Hypersecretion under these conditions might only have been expected in response to such an extraordinary stimulation as the direct massage of the glands or strong galvanization of the secretory nerve, which Stewart found to cause an increased adrenalin output. Indeed the entire lower part of the animal body with many abdominal organs was already in an asphyxial condition at the conclusion of the preparations for securing the blood sample, if I am not mistaken. And again, as stated above, the epinephrin may be produced and discharged into the circulation by other glands than the adrenals.

There is too great a discrepancy between Stewart's results and those of Cannon, who has proved a remarkable increase of adrenalin in the caval blood and sometimes even in the heart blood during asphyxia. Whether this discrepancy is due to the inaccuracy of the methods employed for the estimation of epinephrin or due to the diversity of methods of obtaining the blood sample, is a question which calls for further investigation.

The results of the experiments which I have to report here seem to be rather in accordance with the adrenalin theory.

I have undertaken this work with the object of ascertaining whether the asphyxial blood has any appreciable ability of provoking hyperglycemia when transferred into the circulation of other animals and have obtained a positive result.

Lepine and Boulud (13) have proved the existence of a substance in asphyxial blood which can induce glycosuria when injected into animals. Lepine called this substance "Leucomaïnes diabétogènes." He extracted it from the asphyxial blood of dogs, and could cause a remarkable glycosuria in guinea pigs by injecting this extract. According to his hypothesis, this substance is produced in the blood by the lack of oxygen, and causes the glycosuria by preventing the oxidation of sugar. But the Leucomaïnes diabétogènes is said to be capable of inducing a glycosuria lasting two to three days by only one subcutaneous injection. The asphyxial glycosuria and hyperglycemia do not last so long generally. They usually disappear within five or six hours after the removal of the cause. Only the suffocation with CO-gas is reported to be sometimes followed by glycosuria lasting twenty or thirty hours, but this is probably due to the CO poisoning and not to the asphyxia itself. Hence it is very doubtful whether this substance which Lepine separated from asphyxial blood has anything to do with asphyxial glycosuria. Besides no subsequent investigators have been able to confirm the existence of this substance.

In my experiments rabbits were used as experimental animals. The blood was drawn by heart puncture from rabbits during deep asphyxiation and was injected into the auricular vein of other normal rabbits and then the blood sugar of the injected animals was determined twenty to thirty minutes after the injection.

DESCRIPTION OF TECHNIQUE

The hydroöxylamin method of Momose (14) was used for the determination of blood sugar in the beginning of the work, but in the latter part I was obliged to use some other method because I could not obtain the apparatus for Momose's method. I have chosen, therefore, Benedict's method (15) in place of Momose's.

The principle of Momose's method is to let a measured amount of a given sugar solution react with a boiling copper sulphate solution of a known amount larger than the amount of sugar to be reduced, in a atmosphere of ammonia gas in order to avoid the intervention of atmospheric oxygen and then to titrate the amount of copper left unreduced, by means of a standard solution of hydroöxylamine sulphate. The amount of sugar can then be calculated from the amount of the hydrooxylamine solution used. This method gives a very fair result, though it is a little complicated and needs some training in practice. The reason

for employing hydroöxylamin, is that the reducing power of sugar in an alkaline solution is not strong and it decreases with the decreasing concentration of the metal salt to be reduced. If, therefore, the sugar solution were titrated up to the terminal reaction as in the Pavy's method, we can not get a correct value, but a somewhat larger value is obtained. Hence, in this method, the terminal reaction is determined with hydroöxylamine solution which has a strong reducing power. The blood protein is precipitated by colloidal iron. Momose obtained 0.1383 for the physiological percentage of blood sugar of rabbits. But I got a little lower value, probably owing to the precautions I took in obtaining the blood sample. I chose old rabbits and drew the blood from the auricular vein avoiding agitation of the animals and did not draw more than 6 cc. at one time from one rabbit. The blood was taken uniformly 3 hours after the morning feeding. Some of the results are given below.

RABBIT NUMBER	DATE OF THE DETERMINATION	PERCENTAGE OF SUGAR
N. 1	February 9	0.0960
N. 2	February 9	0.1036
N. 3	February 9	0.1292
N. 4	February 11	0.1264
N. 5	February 11	0.1132
N. 1	February 13	0.1279
N. 4	February 14	0.1008
N. 3	February 14	0.1270
N. 2	February 14	0.1124

Experiment 1. Healthy rabbits were suffocated by pressing the trachea with fingers, taking precautions not to press the carotids and vagus, and as soon as the animals were unconscious the pressing fingers were released and artificial respiration was applied. When the animals had become conscious, once more asphyxiation was brought about in the same way as before, and during this second asphyxia the blood was drawn by heart puncture with a sterile hypodermic needle, into about 0.01 gram of hirudin. (I must offer my heartiest thanks to Professor Tatum of Chicago University for his kindness in giving me the hirudin.)

Asphyxial blood coagulates quite slowly, but it is liable to coagulate within the needle when we wish to reinject the blood into other animals unless an anticoagulant is used.

The asphyxial blood thus taken was introduced immediately into the auricular vein of normal rabbits in which the percentage of blood sugar

had been previously determined. A small portion of the asphyxial blood was left for the determination of sugar.

The blood was drawn 20 to 30 minutes after the injection of the asphyxial blood from the auricular vein of the injected animals and its percentage of sugar was estimated. In case the rabbits did not revive from the first suffocation, the asphyxial blood drawn from the dead animals was used. Many rabbits died when the blood was taken during the second asphyxiation.

The results thus obtained are given below.

NUMBER	WEIGHT OF THE INJECTED RABBITS	SUGAR BEFORE INJECTION	AMOUNT OF THE INJECTED BLOOD	SUGAR OF THE INJECTED BLOOD	FATE OF THE SUFFOCATED RABBITS	SUGAR AFTER INJECTION
	grams	*per cent*	*cc.*	*per cent*		*per cent*
1	2905	0.121	11.5	0.266	Alive	0.135
2	3202	0.090	14.0	0.146	Alive	0.134
3	2650	0.147	12.0	0.279	Alive	0.148
4	2895	0.128	17.0	0.320	Died in second asphyxiation	0.149
5	3430	0.115	10.0	0.208	Alive	0.152
6	2250	0.105	15.0	0.217	Alive	0.178
7	2280	0.123	15.0	0.265	Died in second asphyxiation	0.197
8	2545	0.136	15.0	0.305	Died in first asphyxiation	0.141
9	3160	0.105	18.0	0.196	Alive	0.158
10	2750	0.122	17.0	0.190	Died in first asphyxiation	0.204
11	2455	0.120	19.0	0.283	Died in second asphyxiation	0.172

All animals used in the experiment were tested previously for the existence of isolysin in their blood because I feared that the hemolysis caused by isolysin might induce a kind of partial internal asphyxiation by destroying the red blood corpuscles of the injected animals, which in turn might provoke hyperglycemia, just in the same way as hyperglycemia is believed to arise in CO-poisoning. In order to test for isolysin, a small amount of the blood sample was drawn from the rabbits to be suffocated, the serum was separated and was mixed with the blood corpuscles taken from rabbits to be transfused, kept at 37°C. for one hour, and the result observed.

As will be seen in the table, the blood sugar percentage of rabbits injected with the asphyxial blood, showed an increase almost invariably twenty to thirty minutes after the injection. In no. 10. the increase was very remarkable. In this case the injected asphyxial blood had only 0.190 per cent sugar while the sugar percentage of the injected animal rose from 0.122 to 0.204. This might be considered as an error.

On the other hand it may be perhaps due to the greater sensibility of the injected animal than the suffocated for the substance causing disturbance of the sugar metabolism.

Before concluding that this ability of provoking hyperglycemia is a special property of asphyxial blood, it is necessary to ascertain the influence of normal blood upon the sugar metabolism when introduced into the circulation.

There are not many reports concerning the relationship between the sugar metabolism and the injection of protein. Henderson and Underhill (16) reported that hyperglycemia is caused by peptone injection owing to the acapnia induced by peptone poisoning. Hugh MacGuigan (17) found, on the contrary, hypoglycemia in peptone poisoning and generally in anaphylaxis. He writes in his report that "in making blood transfusion from one animal to another, they have noticed there is a general tendency for the blood sugar of the recipient to fall." But unfortunately he does not furnish the experimental basis for this conclusion and I was therefore uncertain as to the dose of blood transfused and the manner of transfusion and whether his transfusion was iso- or heterotransfusion. It was necessary, accordingly, to undertake the next experiment as control for the experiment already described.

Experiment 2. Blood was drawn from the auricular vein of healthy rabbits very cautiously without causing their agitation. Five to six cubic centimeters of normal blood were obtained from one animal. About 0.01 gram of hirudin was added to each 10 cc. of the blood which was then injected into the auricular vein of other rabbits, in which the blood sugar percentage had been previously determined. The injected animals received, therefore, the normal blood drawn from 2 or 3 other rabbits. The so-called *aderlass* hyperglycemia can not occur by drawing 5 or 6 cc. blood from one animal (18, 19).

Control experiment with normal blood

NUMBER	WEIGHT OF THE INJECTED RABBITS	SUGAR BEFORE INJECTION	AMOUNT OF THE INJECTED BLOOD	SUGAR 20 TO 30 MINUTES AFTER INJECTION
	grams	*per cent*	*cc.*	*per cent*
1	2795	0.112	15	0.112
2	3110	0.125	15	0.137
3	2560	0.122	15	0.137
4	2785	0.098	15	0.124
5	3310	0.102	15	0.090
6	2845	0.124	18	0.115
7	2405	0.135	18	0.135
8	2600	0.122	18	0.104

As will be seen from the table, I could not observe any distinct change of the blood sugar percentage caused by transfusion of the normal blood within the limits of dosage which I used. Such small variations as occurred in no. 4 or no. 9 may be caused by a slight technical failure of determination or by an agitation of the animals which can not be prevented.

This experiment proves also that hirudin had no effect on blood sugar, at least within the dosage I employed.

Thus I believe it certain that the ability of provoking hyperglycemia is a special property of asphyxial blood.

It is a question as to what agent of asphyxial blood this property is due. I carried out a few experiments endeavoring to find out the probable agent. But I could not reach any definite conclusion. In the next experiment I have shown that the excess of sugar contained in the asphyxial blood is not responsible for this ability.

Experiment 3. The injected asphyxial blood had somewhat large amount of sugar as shown in the table, some had 0.305 per cent and some had 0.320 per cent. In 18 cc. of such a blood there will be about 0.035 gram more glucose than the normal amount of sugar in that volume of blood, assuming the physiological percentage of sugar to be 0.12 per cent. If a rabbit is supposed to have 100 cc. blood (weight: blood = 20 : 1), and 0.035 gram sugar was introduced into circulation, this will make 0.035 per cent and the total percentage of the blood sugar of animals injected with the asphyxial blood, therefore, ought to have been 0.155 per cent at most, if the excess of sugar in the asphyxial blood were the sole cause of the rise of sugar percentage in the injected animals. But such calculation as this was not at all applicable as a matter of fact. The increase of sugar found never corresponded to the amount contained in the asphyxial blood. This fact alone is obvious evidence that the hyperglycemia is caused by some other agent than the excess of sugar. But further evidence is advanced in experiment 3 in order to fully eliminate the possibility that the excess of sugar may have disturbed the sugar metabolism of the injected animals.

Normal blood was drawn in the same way as described in the experiment 2, and 0.02 gram glucose was added to each 10 cc. of the blood, which was then injected slowly into the auricular vein of healthy rabbits. If the excess of sugar in the injected blood were the main cause of the hyperglycemia obtained in experiment 1, this normal blood with added glucose should give a similar result. The sugar determination was done with blood taken 20 to 30 minutes after the injection.

The results are as follows:

WEIGHT OF RABBITS	SUGAR BEFORE INJECTION	AMOUNT OF INJECTED BLOOD	SUGAR AFTER INJECTION
grams	*per cent*	*cc.*	*per cent*
3120	0.106	15	0.124
2875	0.130	15	0.135
2640	0.111	15	0.111
2800	0.128	15	0.142
2550	0.122	18	0.112
2925	0.137	18	0.133

As is shown in the table the result was not the same as that obtained with the asphyxial blood. The sugar percentage after injection was almost the same as before. This result agrees well with that of the experiment done by Thanhauser (20), who proved that the normal blood sugar percentage is restored within 15 minutes after the injection of 550 cc. of 7 per cent grape sugar solution into the vena mediana cubiti of man. Kleiner carried out similar experiments with animals and proved that a great part of the injected dextrose is transferred to the tissues and changed in polysaccharides very quickly (21).

Experiment 4. It is a well-known fact that in various kinds of experimental as well as clinical diabetes, the acidity of blood increases. In the asphyxial blood the increase of acidity is partly due to the excess of CO_2, but it has been proved by Araki (22) that lactic acid, oxalic acid, etc., are also responsible for it. This fact has been confirmed by subsequent investigators.

On the other hand, it is a fact beyond doubt that weak acid has an accelerating influence upon the activity of diastase or glycogenolytic ferment (23). Arguing from these facts and supported by other experimental data, many authors believe that the cause of asphyxial glycosuria or hyperglycemia is the excess of CO_2 in the blood (24) or the increase of acidity (25).

The following experiment was, therefore, performed to investigate whether the hyperglycemia, provoked by the injection of the asphyxial blood, is not attributable to the acidity.

According to my experiment, the H-ion concentration of the normal arterial blood, taken from the left ventricle of the rabbit's heart, and that of the venous blood from the auricular vein, when determined by the gas chain method of Michaelis (26), is as follows:

Arterial blood drawn by heart puncture

ROOM TEMPERATURE	MILLIVOLT OBTAINED WITH STANDARD SOLUTION	MILLIVOLT OBTAINED WITH BLOOD	PH
degrees C.			
18	514.8	677.0	7.39
20	515.0	682.2	7.46
20	516.0	680.0	7.42
20	516.0	678.0	7.40
19	515.5	686.0	7.54
Venous blood taken from auricular vein			
20	516.6	668.0	7.21
20	516.1	681.0	7.43
19	516.0	673.3	7.32
20	516.0	675.5	7.34
20	516.5	672.5	7.30

The H-ion concentration of the asphyxial blood obtained in the same way as described in the experiment 1 and determined by the gas chain method, is as follows:

ROOM TEMPERATURE	MILLIVOLT OBTAINED WITH STANDARD SOLUTION	MILLIVOLT OBTAINED WITH BLOOD	PH
degrees C.			
19	516.0	634.0	6.66
20	516.0	634.5	6.64
20	516.6	629.0	6.54
20	516.5	641.0	6.75
20	516.2	652.5	6.94

As mentioned above, the PH of the asphyxial blood was 6.54 to 6.94 while that of the physiological blood was 7.54 to 7.39 (arterial), 7.21 to 7.43 (venous). This acidity may have been the cause of the hyperglycemia in the recipients in our experiment 1. I therefore neutralized the asphyxial blood with alkali carbonate solution, and repeated the experiment with this neutralized asphyxial blood, to investigate whether the hyperglycemia will fail to occur after neutralization of the blood.

In order to neutralize the blood, the H-ion concentration was first determined by the indicator method and then Na_2CO_3 solution (10 per cent) was added to the saline solution used for the indicator method until the PH became 7.5 to 7.6, and then the amount necessary to be added to the asphyxial blood was calculated from the amount required.

The blood thus neutralized was injected into healthy rabbits. (I wish to express here my gratitude for the kindness of Doctor Tashiro of Chicago University for lending me the apparatus for the determination of H-ion concentration.)

The results thus obtained are given below:

WEIGHT OF RABBITS	SUGAR BEFORE INJECTION	AMOUNT OF INJECTED BLOOD	SUGAR AFTER INJECTION
grams	*per cent*	*cc.*	*per cent*
2815	0.135	15	0.172
2310	0.128	18	0.144
2570	0.096	15	0.180
2645	0.137	15	0.188
3105	0.119	15	0.156
2760	0.131	15	0.145
2650	0.122	15	0.127

As cited in the table, I observed that the tendency to promote hyperglycemia remains in the asphyxial blood even when the blood was neutralized. Evidently, therefore, the acidity of the asphyxial blood samples was not responsible for the effect induced.

DISCUSSION

It is shown in this work that asphyxial blood causes a rise in the sugar content of blood when introduced into the circulation of other animals. It can not be stated what products of asphyxia act as the primary cause of asphyxial hyperglycemia. The lack of oxygen may form a primary cause by leading to deficient oxidation of carbohydrates, as Claude Bernald, Dastre, Lepine, Terrey, Araki and others believe, or the excess of CO_2 and the increased acidity may be the primary cause, as Edie, Moore, Roaf, Macleod and others have suggested, or the blood sugar may increase owing to a decrease of the activity of tissue oxidase as Underhill thought, or the asphyxial glycosuria and hyperglycemia may be due to the emotional disturbance or the fear of death, as Bang and Stenström opine, because it is not possible to suffocate animals without causing fear of death. Or it is very possible that all of these factors may combine to give rise to asphyxial hyperglycemia. But however this may be, the asphyxial blood seems to possess in itself hyperglycemic ability. This ability is neither due to the excess of sugar contained in it, nor to its acidity.

In order to explain this experimental fact, the hypothesis that adrenalin is responsible is most acceptable because we do not know at present any other substance than adrenalin in the blood which can give rise to the enhanced sugar content, though, of course, we can not venture to claim that hyperadrenalinemia was proved by our experiment to exist in asphyxia, for some hitherto unknown agent may be discovered in the future to have been responsible for the effect obtained.

SUMMARY

1. The transfusion of the normal blood from rabbits to rabbits has no remarkable effect upon the blood sugar percentage.

2. The transfusion of the asphyxial blood causes a rise in the sugar content of the blood of recipients.

3. The excess of sugar content in the asphyxial blood is not responsible for this increase of sugar percentage in the blood of the recipients,

4. The neutralization of the blood with Na_2CO_3 solution does not abolish this property of asphyxial blood.

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UREA EXCRETION AFTER SUPRARENALECTOMY

GEORGE BEVIER AND A. E. SHEVKY

From the Medical Division of the Stanford University Medical School, San Francisco

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The rate of urea excretion has been shown to be primarily a function of the concentration of urea in the blood (1). But if we divide the urea excretion per hour by the amount of urea in 100 cc. of blood (Addis' ratio, (2)) and thereby get an expression of the rate of excretion per unit concentration, we find that, in rabbits, this rate of excretion per unit concentration presents a rather wide range of variability —in other words, that for any given concentration of urea in the blood the rate of excretion may be either rapid or slow. This variability must be accounted for by assuming the operation of factors other than the concentration of urea in the blood (3) and the quantity of functioning renal tissue (4). It has further been shown that the subcutaneous injection of epinephrin has the same effect as those factors which *increase* the rate of urea excretion for a given blood concentration (5), and that the subcutaneous injection of pituitrin affects this ratio by *depressing* it (6), that is, the rate of urea excretion at any given blood urea concentration is accelerated after the injection of epinephrin and depressed after the injection of pituitrin. By mixing epinephrin and pituitrin in doses of varying proportions it is possible to get a modified effect of either one, or the doses may be so balanced that the ratio is not affected in either direction (7).

These facts have suggested the hypothesis that an epinephrin-pituitrin balance may exist in the blood which can alter the rate of renal activity in the handling of urea. One way in which the existence of such an epinephrin-pituitrin balance can be investigated is by a double suprarenalectomy which should leave the pituitary effect unopposed. Similarly, removal of the hypophysis cerebri should give an unopposed epinephrin effect. The results of a few not altogether satisfactory experiments on the effect of suprarenalectomy were referred to in a previous communication (7). At that time it was not possible to

obtain more data, but we have recently been able to return to the problem and in this paper present the results of more numerous and better planned experiments.

METHODS

Male rabbits were used and the procedure was the same as described previously (1) with the exception that no stomach tube was introduced. Briefly, it consisted of catheterizing the rabbits, which had been kept in the laboratory without food or water since the previous afternoon, at a definite time (9 a.m.) and then collecting the urine by catheterization at the end of the first, second, third and fifth hours. At the middle of each interval 1 cc. samples of blood were obtained from the ear veins. The urea determinations were made with Marshall's urease method, using for the urine the titration method with modifications as detailed by Addis and Watanabe (8) and for the blood the aeration method with the refinements introduced by Barnett (9).

Using this technique the excretion of urea per hour for the various periods, and the corresponding blood urea concentrations were determined, first, for a group of *normal* animals. By dividing the number of milligrams of urea excreted per hour by the number of milligrams of urea in 100 cc. of blood during the same hour, we get the "ratio" (2) or the rate of excretion per unit blood concentration. These values have also been tabulated in our data.

Bilateral lumbar incisions were then made on some of the animals, exposing and manipulating the suprarenals, but not actually removing them, to ascertain any effect of the operation itself, or of the anesthetic, on our ratio curve.

Subsequently the suprarenal glands of these rabbits were removed through lumbar incisions under ether anesthesia. At first we attempted to remove both glands on the same day and immediately follow the operation by a determination of the rate of urea excretion in the same manner as we had done on the normal animals. These animals often died in an extremely exhausted condition within a few hours and often during the course of the experiment. Often kidney function was almost completely depressed in these dying animals. Evidently such a condition of shock would be accompanied by renal disturbances not representative of the true effect of suprarenal removal. After considerable experimenting we concluded that the removal of one gland at a time with an interval of several days between operations and a rest of a day after the final operation before we commenced the

procedure of collecting the urine and blood, gave the most dependable results, coinciding with the findings of others (10), (11). A large percentage of our animals survived, especially after we became more expert in the technique of the operation. The rabbits were under anesthesia from 20 to 25 minutes. On the day following the final operation they appeared to be about as lively and vigorous as the normal animals. The rate of excretion was determined by our procedure then, and on several subsequent occasions.

THE SUPRARENAL GLANDS IN THE RABBIT

Since tissues similar to those found within the suprarenal capsules are found elsewhere in the bodies of some animals, the question arises as to just what we remove when we excise these capsules from the rabbit. The comparative anatomy has been discussed quite fully, and also some of the general results of suprarenalectomy, by Biedl (12) and earlier by Tizzoni (13). Both the cortical and the medullary tissues of the suprarenal may occur separately outside of the capsules in the rabbit as in nearly all of the animals having definite, separate, isolated glands. Medullary or chromaffine cells are found in the ganglia of the abdominal sympathetic and in the carotid ganglion. Stilling (14) observed that extirpation of one gland in the rabbit was followed by great hypertrophy of the other gland and of any remnants of the glands which happened to be left at the time of operation. Also that accessory suprarenals are frequently found after ablation of one gland, probably due to a marked proliferation of the isolated patches of this tissue which occur along the vena cava and in other parts. Fulk and Mac'eod (15) have shown that retroperitoneal chromaphil tissue is the same as that of the suprarenal capsules, and that extracts of it have the same reactions as the medullary tissue.

DATA

In figure 1 we have plotted curves (from table 1) showing the average excretion of urea in milligrams, the average concentration of urea per 100 cc. of blood, and the average ratio for each of the periods of our experiment, for a series of twenty normal animals before operation. As has been previously mentioned, the ratio =

$$\frac{\text{Urea excreted per hour in mgm.}}{\text{Mgm. of urea in 100 cc. of blood.}}$$

TABLE 1

Normal animals

RABBIT	UREA EXCRETED PER HOUR IN GRAMS FOR THE FOUR PERIODS				UREA IN 100 CC. OF BLOOD IN GRAMS				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
93	0.020	0.039	0.058	0.075	0.045	0.043	0.051	0.058	0.45	0.91	1.15	1.29
121	0.032	0.038	0.017	0.042	0.033	0.032	0.030	0.035	0.91	1.19	0.39	1.21
121	0.026	0.032	0.027	0.029	0.033	0.030	0.030	0.027	0.80	1.07	0.88	1.09
122	0.013	0.013	0.021	0.028	0.020	0.023	0.021	0.022	0.67	0.58	1.02	1.27
127	0.084	0.112	0.116	0.123	0.105	0.143	0.145	0.148	0.79	0.80	0.80	0.83
134	0.008	0.059	0.066	0.069	0.030	0.033	0.030	0.036	0.27	1.78	2.18	1.93
137	0.023	0.013	0.010	0.037	0.032	0.032	0.035	0.036	0.69	0.41	0.33	1.00
138	0.026	0.024	0.038	0.035	0.042	0.042	0.042	0.045	0.65	0.57	0.91	0.80
139	0.024	0.023	0.028	0.030	0.034	0.035	0.040	0.039	0.69	0.67	0.70	0.79
142	0.005	0.007	0.022	0.024	0.031	0.027	0.057	0.041	0.17	0.24	0.40	0.60
51	0.009	0.029	0.035	0.051	0.057	0.048	0.042	0.051	0.15	0.60	0.84	1.00
51	0.046	0.076	0.085	0.103	0.039	0.048	0.048	0.040	1.17	1.59	1.74	2.60
52	0.023	0.023	0.013	0.046	0.049	0.035	0.052	0.046	0.46	0.65	0.24	1.00
52	0.018	0.026	0.054	0.086	0.033	0.037	0.036	0.039	0.53	0.72	1.51	2.20
53	0.031	0.032	0.044	0.060	0.027	0.037	0.043	0.045	1.14	0.88	1.03	1.34
56	0.029	0.027	0.021	0.039	0.033	0.038	0.040	0.030	0.89	0.71	0.53	1.30
58	0.024	0.028	0.031	0.039	0.038	0.033	0.028	0.035	0.63	0.86	1.12	1.12
60	0.004	0.004	0.015	0.017	0.037	0.034	0.036	0.036	0.12	0.13	0.42	0.47
61	0.007	0.004	0.016	0.015	0.022	0.024	0.027	0.038	0.34	0.19	0.58	0.41
62	0.013	0.009	0.013	0.015	0.024	0.024	0.021	0.030	0.56	0.38	0.64	0.50
	0.465	0.618	0.730	0.963	0.764	0.798	0.855	0.877	12.08	14.93	17.41	22.75
Average	0.023	0.031	0.037	0.048	0.038	0.040	0.043	0.044	0.61	0.75	0.87	1.14
Probable error....................................									±0.19	±0.27	±0.33	±0.38

It will be observed that the ratio becomes higher—that the rate of excretion of urea per unit concentration of urea in the blood increases—as the experiment progresses, being highest in the last hour. It will be convenient for us, in this discussion, to refer to this tendency for the curve to become higher in the successive periods, as the "epinephrin effect" since it was found to be most marked after the injection of epinephrin (5).

Opposite the curves for normals are drawn similar curves representing the averages of seventeen experiments after both glands had been removed. The ratio curve here does not rise, that is, we do not find

the "epinephrin effect" that occurs in normal animals. The data for this curve are tabulated in table 2.

Figures 3 and 4 are taken from papers already referred to (3), (5) and are introduced for comparison. They show the effects, respectively, of

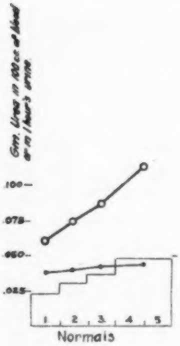


Fig. 1

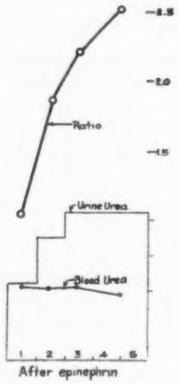


Fig. 2

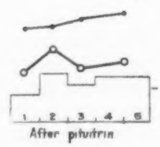


Fig. 3

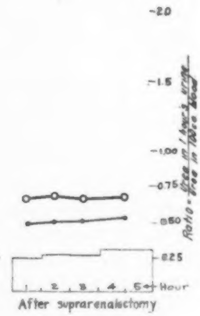


Fig. 4

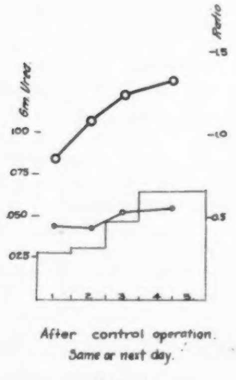


Fig. 5

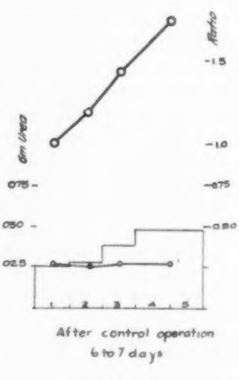


Fig. 6

injecting, subcutaneously, 0.25 cc. Parke, Davis & Company adrenalin at the beginning of each hour, and 0.25 cc. of the same company's pituitrin. The ratio curve rises most markedly after epinephrin and is depressed after pituitrin.

Figure 5 gives the curves for the average of six experiments (table 3) performed on the same day as the *control operation* when the glands were exposed and manipulated but not removed, and figure 6 shows the results of determinations made on some of these same animals on the sixth and seventh days after the control operation. Evidently

TABLE 2

A compilation of all observations made after both suprarenal glands had been removed. They were made on the day following the removal of the final gland or later.

RABBIT	UREA, IN GRAMS, EXCRETED PER HOUR				GRAMS OF UREA IN 100 CC. OF BLOOD				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
134	0.034	0.029	0.035	0.039	0.043	0.042	0.045	0.047	0.78	0.68	0.77	0.82
134	0.026	0.024	0.032	Lost	0.024	0.024	0.026	0.027	1.10	1.00	1.25	
138	0.033	0.040	0.035	0.047	0.036	0.048	0.045	0.039	0.92	0.83	0.80	1.20
51	0.037	0.040	0.036	0.047	0.036	0.036	0.036	0.040	1.03	1.10	1.00	1.15
52	0.010	0.019	0.009	0.008	0.043	0.042	0.045	0.042	0.24	0.45	0.20	0.18
58	0.010	0.021	0.013	0.019	0.030	0.037	0.040	0.038	0.25	0.57	0.32	0.50
60	0.012	0.007	0.001	0.011	0.057	0.056	0.051	0.048	0.21	0.12	0.02	0.23
61	0.056	0.052	0.056	0.049	0.045	0.045	0.036	0.042	1.27	1.15	1.53	1.16
62	Lost	0.031	0.032	0.035	0.045	0.042	0.039	0.042		0.75	0.81	0.83
51	0.049	0.041	0.060	0.063	0.047	0.043	0.048	0.051	1.04	0.96	1.25	1.23
52	0.063	0.055	0.075	0.068	0.076	0.081	0.085	0.092	0.82	0.68	0.88	0.74
58	0.010	0.008	0.001	0.001	0.019	0.031	0.025	0.029	0.55	0.26	0.04	0.04
60	0.024	0.017	0.019	0.014	0.037	0.042	0.038	0.039	0.65	0.41	0.51	0.37
61	0.010	0.011	0.009	0.008	0.032	0.030	0.029	0.027	0.33	0.35	0.31	0.29
62	0.016	0.020	0.007	0.001	0.072	0.078	0.082	0.096	0.23	0.23	0.08	0.02
134	0.029	0.047	0.040	0.065	0.028	0.024	0.027	0.034	1.05	1.96	1.50	1.90
138	0.029	0.035	0.035	0.053	0.042	0.042	0.045	0.042	0.68	0.84	0.77	1.27
	0.448	0.497	0.495	0.528	0.712	0.743	0.742	0.775	11.15	12.34	12.04	11.93
Average	0.028	0.029	0.029	0.033	0.042	0.044	0.044	0.045	0.70	0.73	0.71	0.74
Normal.	0.023	0.031	0.037	0.048	0.038	0.040	0.043	0.044	0.61	0.75	0.87	1.14
Difference between ratios									+0.09	−0.02	−0.16	−0.40

any differences in the ratio curves from the normal, due to the anesthetic or to the operation per se, lie entirely within the range of variability of the series and are not measureable. We are, then, justified in interpreting the differences in the curves before and after suprarenalectomy as due to the loss of the glands.

TABLE 3

These data were obtained on the same day as the control operations, when lumbar incisions were made and the glands exposed and manipulated but not removed. The purpose is to ascertain the effect of the anesthetic and the operation per se, as differentiated from the removal of the suprarenals.

RABBIT	UREA EXCRETED PER HOUR IN GRAMS				UREA IN 100 CC. OF BLOOD, IN GRAMS				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
93	0.007	0.007	0.024	0.054	0.045	0.045	0.075	0.060	0.15	0.15	0.32	0.89
116	0.049	0.043	0.068	0.067	0.043	0.041	0.042	0.039	1.13	1.06	1.62	1.72
117	0.046	0.051	0.062	0.049	0.030	0.028	0.030	0.043	1.51	1.81	2.08	1.14
121	0.010	0.021	0.035	0.050	0.036	0.038	0.045	0.044	0.29	0.56	0.77	1.13
122	0.037	0.038	0.043	0.099	0.100	0.100	0.108	0.110	0.37	0.38	0.40	0.90
134	0.020	0.023	0.048	0.072	0.012	0.009	0.021	0.034	1.65	2.53	2.24	2.10
Average.....	0.028	0.031	0.047	0.065	0.044	0.043	0.053	0.055	0.85	1.08	1.24	1.32

TABLE 4

Observations made on the sixth and seventh days after the control operation. In both this table and table 3 it will be noted that the values of the ratio for the successive periods continually increase after the manner of normal animals

RABBIT	GRAMS OF UREA EXCRETED PER HOUR				GRAMS OF UREA IN 100 CC. OF BLOOD				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
93	0.012	0.014	0.044	0.065	0.033	0.033	0.036	0.033	0.36	0.44	1.23	1.97
116	0.016	0.021	0.019	0.023	0.020	0.015	0.017	0.019	0.81	1.42	1.13	1.20
117	0.044	0.044	0.052	0.055	0.024	0.026	0.027	0.026	1.84	1.70	1.93	2.03
Average.....	0.024	0.027	0.038	0.047	0.026	0.025	0.027	0.026	1.00	1.19	1.43	1.73

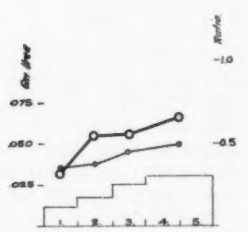
Nine observations on rabbits after the removal of the right gland and before the removal of the left one, gave averages shown in figure 7 and table 5. The ratio curve presents a distinct upward tendency but is slightly less pronounced than in the normal animals. These findings are introduced merely for the sake of completeness; they seem to indicate that the remaining gland is ample to supply the needs of the body.

Figures 8 and 9 are constructed, respectively, from observations made from 24 to 48 hours after the removal of the final gland, and

TABLE 5

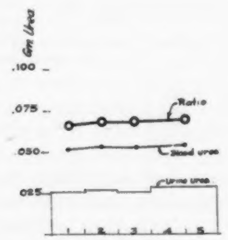
These observations were made on the same day or the next day after the removal of the right suprarenal gland and before the excision of the left gland, in order to ascertain the renal behavior of an animal with only one suprarenal. If there is any effect it is too small to be definite

RABBIT	UREA EXCRETED PER HOUR IN GRAMS				UREA IN 100 CC. OF BLOOD, IN GRAMS				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
127	0.002	0.006	0.014	0.014	0.046	0.046	0.054	0.032	0.05	0.14	0.25	0.32
137	0.002	0.004	Lost	0.014	0.018	0.008	0.023	0.039	0.08	0.56		0.36
138	0.009	0.009	0.024	0.017	0.060	0.054	0.064	0.075	0.15	0.17	0.37	0.24
139	0.003	0.020	0.014	0.024	0.039	0.036	0.038	0.049	0.08	0.54	0.37	0.50
141	0.002	0.003	0.007	0.018	0.030	0.033	0.033	0.037	0.07	0.09	0.23	0.46
143	0.027	0.006	0.030	0.035	0.039	0.042	0.047	0 066	0.70	0.14	0.64	0.53
51	0.005	0.043	0.030	0.051	0.015	0.025	0.042	0.048	0.30	1.78	0.95	1.07
52	0.019	0.019	0.030	0.050	0.033	0.046	0.049	0.056	0.58	0.42	0.62	0.89
62	0.035	0.044	0.041	0.051	0.039	0.040	0.042	0.036	0.88	1.07	0.99	1.40
Average.....	0.012	0.017	0.025	0.030	0.035	0.037	0.044	0.049	0.32	0.54	0.55	0.64



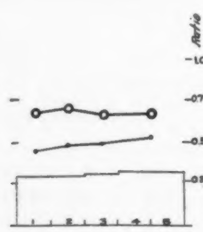
Unilateral suprarenalectomy.

Fig. 7



24 to 48 hours after final operation

Fig. 8



5 to 8 days after final operation.

Fig. 9

from 5 to 8 days after the last operation. There is no difference between these curves, indicating that if there is a readjustment of the "balance" after the loss of the suprarenals, it does not occur very soon.

Figure 10 is a graphical summary comparing the present results with some previously obtained. The base line represents the average ratio during the first hour for a group of normal animals. It is the

average of observations on fifty-seven rabbits and its value is 0.69. The rest of the curve is obtained by computing the percentage of this quantity by which the ratio is increased or decreased during the following hours of the experiment and under the varying conditions of it. In the curve for "normals" we have the percentage by which the ratio was increased after the first hour. The curve for "epinephrin" was

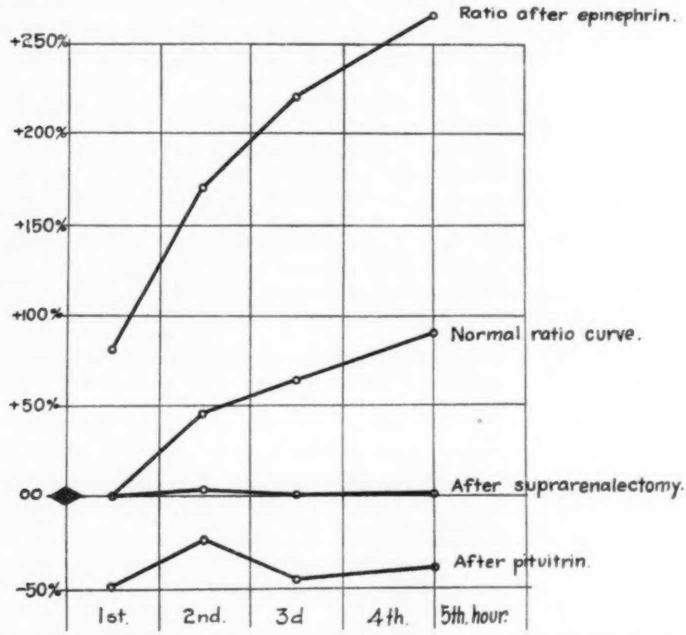


Fig. 10. Chart showing the percentage increase or decrease in the average "ratio" for the various periods of the experiments and under the various conditions listed, compared with the average "ratio" for the first period obtained from 57 normal animals. See table 8.

obtained from data recorded by Addis, Barnett and Shevky (5) and shows the marked increase obtained after the subcutaneous injection of 0.25 cc. Parke, Davis & Company adrenalin at the beginning of each hour, in percentage of the initial ratio for the first hour for animals under normal conditions. It will be noted that there was an increase in the average ratio of about 80 per cent as the result of the first injec-

TABLE 6

In this table we have collected those observations which were made from twenty-four to forty-eight hours after the excision of the final suprarenal capsule. It may be compared with the next table in which we have collected observations made from five to eight days after the removal of the last gland

RABBIT	UREA EXCRETED PER HOUR IN GRAMS				UREA IN 100 CC. OF BLOOD				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
134	0.034	0.029	0.035	0.039	0.043	0.042	0.045	0.047	0.78	0.68	0.77	0.82
134	0.027	0.024	0.033	Lost	0.024	0.024	0.026	0.027	1.10	1.00	1.25	
138	0.033	0.040	0.035	0.047	0.036	0.048	0.045	0.039	0.92	0.83	0.80	1.20
51	0.037	0.040	0.036	0.047	0.036	0.036	0.036	0.040	1.03	1.10	1.00	1.15
52	0.010	0.019	0.010	0.008	0.043	0.042	0.045	0.042	0.24	0.45	0.20	0.18
58	0.010	0.021	0.013	0.019	0.039	0.037	0.040	0.038	0.25	0.57	0.32	0.50
60	0.012	0.001	0.001	0.011	0.057	0.056	0.051	0.048	0.21	0.12	0.02	0.23
61	0.056	0.052	0.056	0.049	0.044	0.045	0.036	0.042	1.27	1.15	1.53	1.16
62	Lost	0.032	0.032	0.035	0.045	0.042	0.039	0.042		0.75	0.81	0.83
	0.219	0.258	0.251	0.255	0.367	0.372	0.363	0.365	5.80	6.65	6.70	6.07
Average.....	0.027	0.029	0.028	0.0 2	0.041	0.041	0.040	0.041	0.73	0.75	0.74	0.76

TABLE 7

Data obtained from five to eight days after the excision of the last suprarenal capsule

RABBIT	UREA EXCRETED PER HOUR IN GRAMS				UREA IN 100 CC. OF BLOOD, IN GRAMS				RATIO			
	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours	1st hour	2d hour	3d hour	4th and 5th hours
51	0.049	0.041	0.060	0.063	0.047	0.043	0.048	0.051	1.04	0.96	1.25	1.23
52	0.062	0.055	0.075	0.068	0.076	0.081	0.085	0.092	0.82	0.68	0.88	0.74
58	0.011	0.008	0.001	0.001	0.019	0.031	0.025	0.029	0.55	0.26	0.04	0.04
60	0.024	0.017	0.020	0.014	0.037	0.042	0.038	0.039	0.65	0.41	0.51	0.37
61	0.010	0.010	0.009	0.008	0.032	0.030	0.029	0.027	0.33	0.35	0.31	0.29
62	0.016	0.020	0.007	0.002	0.072	0.078	0.082	0.096	0.23	0.23	0.08	0.02
.34	0.029	0.047	0.041	0.064	0.028	0.024	0.027	0.034	1.05	1.96	1.50	1.90
	0.201	0.198	0.213	0.220	0.311	0.329	0.334	0.368	4.67	4.83	4.57	4.55
Average.....	0.029	0.029	0.030	0.032	0.044	0.047	0.048	0.052	0.67	0.69	0.65	0.66

tion of adrenalin and that this became greater as the hours passed. The curve for pituitrin shows that the subcutaneous injection of pituitrin at the beginning of each hour depressed the rate of excretion per unit blood concentration. Similarly our results after suprarenalectomy show that the progressive increase detected in normal animals with intact glands, or after the injection of epinephrin, does not occur.

It might be noted that the urea concentration in the blood remains fairly constant under the various conditions of the experiments. It was only in the moribund animals after simultaneous double suprarenalectomy that we found an increase in the level of blood urea similar to that described by Marshall and Davis in cats (16). When one gland was removed at a time the rabbits remained more normal.

TABLE 8

	HOURS			
	1	2	3	4 to 5
Normals: average of 57 animals..........	0.69	1.01	1.13	1.31
Percentage increase over first hour...	00	46.0%	64.0%	90.0%
After epinephrin (28 animals)............	1.04	1.86	2.21	2.52
Percentage increase over normals....	80.0%	170.0%	220.0%	265.0%
After pituitrin (9 animals)...............	0.25	0.52	0.38	0.43
Percentage decrease.................	−49.0%	−25.0%	−45.0%	−38.0%
After suprarenalectomy (17 animals).....	0.70	0.72	0.70	0.71
Percentage increase.................	1.0%	4.0%	1.0%	3.0%

The normal ratio for the first hour of the experiment for a group of fifty-seven rabbits is 0.69. In the second hour it is 1.01 or an increase of 46 per cent and so on for the rest of the periods. After a subcutaneous injection of epinephrin we get an average ratio of 1.04 for the first period or an increase of 80 per cent over the first period excretion for normal animals.

DISCUSSION

Normal rabbits present a progressive increase in the rate of urea excretion during the consecutive periods of our experiment, so that at the end the rate was nearly twice as great as it was in the beginning, in spite of the fact that the urea concentration in the blood remained practically constant. This means, of course, that the kidneys did not have more to do, but performed what they did have to do at a more

rapid rate. The conditions of the experiment were the same at the end as at the beginning except that the animals had undergone considerable handling and discomfort.

The progressive increase found in normal animals may be greatly accentuated by the subcutaneous injection of epinephrin, and may be prevented, or even a progressive decrease may be obtained, by the injection of pituitrin. It has been shown that injections of epinephrin and pituitrin in varying proportions may affect the rate of urea excretion in mutually antagonistic directions and that each may neutralize the effect of the other. And on the basis of these data the hypothesis was advanced that a possible balance between the secretions of the suprarenals and of the hypophysis, in the blood, may be a factor in determining the state of renal activity (7).

The fact that the removal of the suprarenal glands affected the form of the ratio curve in a manner remarkably similar to that produced by the subcutaneous injection of pituitrin (figs. 2 and 4) strongly suggests the existence of such a balance—the effect of suprarenalectomy being an unbalanced pituitary effect.

Marshall and Davis (16) have observed in cats a similar decrease in the rate of excretion of urea, creatinin and chlorides, with which we, of course, agree. Motzfeldt (17) found that the extract or secretion of the posterior lobe of the hypophysis had a strong antidiuretic effect on rabbits, which was most pronounced after 2 to 4 hours. Rees (18) noted that pituitary extracts delay diuresis after ingested water, for 7 or 8 hours, but do not alter the 24-hour volume.

The depression recorded after ablation of the glands is less marked than after the injection of an optimum dose of pituitrin. This is as might be expected, for the excision of the suprarenal capsules has only removed *most*, not all, of the medullary tissue, as previously pointed out. Furthermore, there has been no stimulus to call forth a maximum pituitrin effect, and only the normal amount of pituitary secretion is probably present in the blood. This causes, however, a definite "pituitary effect" for it is not balanced by the full normal suprarenal secretion.

We attribute the progressive increase in the rate of excretion, in spite of constant blood urea concentration, which we found in all normal rabbits during the successive periods of the experiments, to a gradual increase in the rate of secretion of epinephrin from the suprarenal glands. After the removal of the glands such an increase of epinephrin in the blood, of course, could not occur.

CONCLUSIONS

1. The removal of the suprarenal glands in rabbits is followed by a depression of the rate of urea excretion by the kidneys.

2. The form of the curve obtained by plotting the ratio between the urea excreted per hour and the concentration of urea in the blood, for the various intervals of the experiment, is modified after suprarenalectomy in a manner strikingly like that obtained by the subcutaneous injection of optimum doses of pituitrin, and in a manner contrary to that obtained after the injection of epinephrin.

3. It is suggested that these findings support the hypothesis that an epinephrin-pituitrin balance exists in the blood which may regulate the rate of kidney function, the results obtained after suprarenalectomy exhibiting a pituitary effect unopposed by the normal secretion of the suprarenals.

We wish to acknowledge our indebtedness to Dr. Thomas Addis for his valuable suggestions and for his kindly interest, which has always been most stimulating.

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POSTURE-SENSE CONDUCTION PATHS IN THE SPINAL CORD

A Preliminary Report

EUGENE S. MAY AND JOHN A. LARSON

From the Rudolph Speckels Physiological Laboratory of the University of California

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Exact knowledge is lacking as to how impulses mediating posture-sense are conducted in the spinal cord. These, with such other afferent impulses as muscle-sense, deep-sensibility and others of the sensation-complex are supposed to travel upward, without decussation, in the dorsal and lateral columns of the spinal cord. Decussation of these fibers takes place in the medulla superior to the pyramidal decussation. To obtain more definite information as to the manner of transmission of posture-sense impulses in the spinal cord, we have applied to our problem the animal behavior method described by O. Kalischer[1] in his experiments on audition. By means of a strong "hunger-motif" this investigator trained dogs to discriminate between various tones, permitting them to take food only when the correct or "training-stimulus" was presented. Dogs were trained daily over a period of weeks until they perfectly discriminated the "feeding-stimulus" from any other. This "feeding-stimulus" was always the same tone sounded on the organ. After they had learned the "lesson" the animals were operated and an area of the cerebral cortex was destroyed. After complete recovery from the shock of the operation the dogs were again critically tested to determine their ability to perfectly differentiate the "feeding-stimulus" from other stimuli. If discrimination by the animal was still perfect, conclusion was drawn that the center for reception of the impulse was not destroyed. Likewise the converse was held true: that lost or confused discrimination indicated destruction of a specific center.

In our experiments we applied the same principle to tracts in the spinal cord. A dog was trained to take food only when the right hind

[1] Sitzungsb. d. Königl. Preus. Akad. d. Wissensch., 1907, x.

leg was held in a certain position,—that of rigid extension backward; and to refuse food when the same foot was held in any other position. To eliminate possible habit formation by the dog to the stimulus of pressure on the leg, in both phases of the training (extension and flexion) the dog's foot was subjected to an equal pressure by the hand of the operator. Unconscious cues and helps were carefully eliminated. In fact, however, due to the extreme hunger of the animal, it would have been almost impossible to distract his attention from the problem. Grown dogs of all breeds were used but the sharp-nosed type served our purpose best. The dogs were never petted, spoken to nor allowed to commingle. In this way was developed a state of lonesomeness and eagerness for companionship which made them tractable to training and eager for work.

The exact procedure was as follows: The dogs were placed in clean cages and allowed no food for some days. Then by means of the strong "hunger-motif" developed they were taught to leave their cages, to mount three steps to the experiment table, to take a certain position and to wait there until fed. Then the dog's right hind leg was grasped by the operator and extended rigidly backward. A cube of cooked meat was then placed before the dog which he was allowed to take during this phase of the training. This backward extension furnished the "feeding-stimulus" for this animal. Next, the foot was placed in position of rest by the operator, and another cube of meat was offered the dog. During this phase of training the dog was not allowed to seize the particle of food. Only during the first days of the training was it necessary for the operator to interpose his hand between the dog's muzzle and the cube of meat. The dog soon learned when to take food and when to leave it. Punishment was *never* given for mistakes. In our experience such treatment of the dog rendered him unfit for training. As part of the training the animals were taught to return to their cages after feeding. Each daily lesson lasted about eight or ten minutes during which time about fifty equal-sized cubes of meat were fed to the dog. Great care was taken not to impair the dog's "hunger-motif," either by over-feeding at the daily lesson or by feeding between lessons. The dogs remained healthy during the experiment but became somewhat emaciated.

The following precautions were taken in our training experiments:

1. Dogs were never petted, spoken to nor punished throughout the course of the training.

2. Dogs were never over-fed nor fed at any other time than training time. In this way a strong "hunger-motif" was maintained. This is the key to the experiment and eliminates such distracting factors as inattention, indifference or fatigue.

3. Duration of time of stimuli was the same and the order of presentation varied by daily rearrangement. This precaution was taken to prevent rhythmic habit formation which might occur if the order of presentation of stimuli were left to chance.

The dogs were considered perfectly trained after they had been taught to differentiate without error the "feeding-stimulus" from all others. They were tested with the "feeding-stimulus" and other stimuli fifteen to thirty times at the daily lesson over a period of two or three weeks. After they were found perfect, the spinal cord was hemisected on the right side about the level of the first thoracic vertebra. Laminectomy was done under ether anesthesia and the cord carefully and completely exposed. Then the dura was incised and the wound packed for a few minutes. After a dry field was secured the cord was carefully hemisected.

After recovery from the shock of the operation, usually the second or third day, discrimination tests were again undertaken, similar to those used during the training of the animal. These tests were carried out over a period of from three to six weeks and were very satisfactory because of the prompt and decided responses of the animals to the stimuli employed. Careful notes on the behavior of the animals were made and will form the basis of full protocols in a later paper.

At the end of six weeks the dogs were killed, the cords removed and the gross hemisection noted. The cords were then preserved in Müller's fluid for histological study of the degenerations. Marchi's method will be used.

SUMMARY

Dog 1. Trained to accept food with right hind leg rigidly extended. *Right* hemisection of cord about level of first thoracic vertebra. Responses to posture-tests in right hind leg prompt and decided. Motor paralysis right hind leg complete. Pain sense lost in right hind leg.

Dog 2. Trained like dog 1. *Right* hemisection of cord about the level of the last thoracic vertebra; and two months later another *right* hemisection of cord about the level of the first lumbar vertebra. Responses to posture-tests in right hind leg prompt and decided. Motor paralysis right hind leg complete. Pain sense lost in right hind leg.

Dog 3. Trained like dogs 1 and 2. *Right* hemisection of cord at level of first thoracic vertebra and two months later *left* hemisection at level of first lumbar vertebra. Responses to posture-tests in right hind leg prompt and decided. Motor paralysis of both hind legs complete.

It was suggested that perhaps the animal was acting upon cutaneous stimuli alone; to eliminate this factor we blocked the cutaneous nerves of the right hind extremity of one animal by injecting a 2.0 per cent solution of cocaine into the skin close to the trunk. Pain sense was then tested with a red hot wire and the animal made no response. The response was unmistakable when the left side was tested.

CONCLUSIONS

1. That decussation of *part* of the fibers mediating posture-sense impulses occurs within the cord.

2. That some of the impulses mediating posture-sense probably travel back and forth across the cord at different levels by short association fibers.

STUDIES ON THE REGULATION OF THE BLOOD DIASTASE

B. FUJIMOTO

From the Forensic-Medical Institute of the Imperial University at Tokyo

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Wohlgemuth (1) reported that the diastase content of the blood is very stable and not influenced by feeding or administration of certain drugs (adrenalin, morphine, etc.), which have a marked effect upon its sugar content. The investigations reported in this paper were carried out in order to determine, if possible, how the blood diastase is regulated.

Clerc and Loeper (2) and Gould and Carlson (3) observed that the ligation of the pancreatic ducts is followed by an increased diastatic activity in the blood serum. They assumed this to be absorbed amylopsin. Otten and Galloway (4) and King (5) found, on the other hand, that the blood diastase sinks rapidly after complete pancreatectomy. The pancreas is, therefore, regarded as the chief source of the blood diastase.

Wohlgemuth (6) stated that the diastatic activity of the blood serum in the portal vein is stronger than that in the hepatic vein. Schlesinger (7) also found that the diastatic power of the blood serum in the pancreatic vein was two or three times stronger than that in the mesenteric vein or the peripheral blood vessel in some cases. Wohlgemuth, in discussing this report of Schlesinger, states that some difference in the diastatic power can be found between the blood in the portal and peripheral veins but not between the blood in the portal and pancreatic veins. Thus it may be said that the diastatic substance passes from the pancreas into the liver and is there mixed with the blood and lymph, its output into the blood being regulated by the liver.

The diastase content of the blood in the portal vein compared with that in the peripheral blood vessel. We have compared the diastase content of the blood in the portal vein with that in the peripheral blood vessel (table 1). In our experiments guinea pigs were always employed. For the estimation of diastase, we have employed Wohlgemuth's

method as modified by Inoue (8). The results of the digestion were seen after incubating for 30 minutes in a water bath at 38°C.

In table 1 we see that the diastase content of the blood in the portal vein varies considerably and that sometimes it is larger than that in the peripheral blood vessel. These results confirm the reports of Wohlgemuth and Schlesinger. Hence it can be said with certainty that the diastase content of the blood is regulated by the liver.

The influence of hepatotoxin upon the diastase content of the blood. Next we have undertaken to injure the liver-cells by parenteral administration of hepatotoxin to disturb the regulation of diastase in the liver.

TABLE 1

The diastase content of the blood serum from the portal vein and carotid artery

GUINEA PIG NUMBER	DIASTASE $D\frac{30'}{38°}$		REMARKS
	Carotid	Portal vein	
21	185	150	
55	125	150	After feeding
56	250	185	After feeding (V. mesenter. 250)
57	215	215	
58	125	150	
59	125	125	

To a series of test tubes there were added increasing amounts of the blood serum and a constant dose of the amylum solution. In our experiments the amounts of the blood serum were so graduated that the diastatic activity in each test tube, when the amylum in it is completely digested, shows each 75, 85, 95, 105, 125, 150, 165, 185, 215, 250, 300.

Though Karsner and Aub (9) have brought forth contradictory findings against the view of Delezenne (10), who had affirmed the organ specificity of hepatotoxin, I have recently proved (not yet published) that hepatotoxin acts specifically on the liver-cells and destroys their normal function. Hence the hypothesis that, if the liver is truly a regulator of the blood diastase, the latter may be influenced by the administration of the hepatotoxin.

The hepatotoxin used in our experiments was obtained by immunizing rabbits with emulsions of the liver of the guinea pig. The intraperitoneal injections were repeated twice. Various doses of hepatotoxin thus obtained were injected intraperitoneally in guinea pigs and the diastase content of the blood was examined repeatedly. The blood was always obtained from the ear. The results are shown in table 2.

As we see in table 2, the diastase content of the blood sinks rapidly after injection of the hepatotoxin and then rises in a few days to its normal value.

From these results we are able to say that the liver cells which regulate the diastase content in the blood were intoxicated by hepatotoxin.

The effect of pancreatotoxin upon the diastase content of the blood. The foregoing experiments were accompanied by the following tests in order to see if other organ toxins can also influence the diastatic activity of the blood. Pancreatotoxin was prepared by immunizing rabbits with emulsions of the pancreas of the guinea pig. The administration of the pancreatotoxin may cause a degeneration of the pancreatic cells. Hence supposing that the pancreatotoxin would influence the production of diastase in the pancreas, we have examined the diastatic activity of the blood after the injection of this toxin (see table 3).

TABLE 2

The diastatic activity of the blood in the peripheral blood vessel before and after the injection of hepatotoxin

GUINEA PIG NUMBER	BODY WEIGHT	AMOUNT OF HEPATOTOXIN INJECTED INTO PERITONEAL CAVITY	DIASTATIC ACTIVITY $D^{30'}_{38°}$							
			Before injection	Day after injection of hepatotoxin						
				3–4 (hours)	1	2	3	4	5	6
	grams									
5	630	3	165			105	125	125		150
29	570	3	165	125	105					165
31	500	3	150	125	105					125
7	600	4	185	150	125		150		165	
9	415	4	165	125	105		165			
10	530	4	165	125	150		150			
47	730	8	105		80	105		105		125
46	590	10	150		95	105		105		165

As we can see in table 3, no appreciable change in the diastase content of the blood in the peripheral blood vessel was observed after injection of the pancreatotoxin, except in two guinea pigs, which received large doses (8 or 10 cc.). Such a large amount of this toxin was near to the lethal dose for guinea pigs. Therefore, it is assumed that the decrease in the diastase content in these two animals was probably caused by an intoxication of the liver cells.

TABLE 3

The diastatic activity of the peripheral blood before and after injection of pancreatotoxin

GUINEA PIG NUMBER	BODY WEIGHT	AMOUNT OF PANCREATOTOXIN INJECTED INTO PERITONEAL CAVITY	DIASTATIC ACTIVITY $D_{38°}^{30'}$							
			Before injection	Day after injection of pancreatotoxin						
				3-4 (hours)	1	2	3	4	5	6
	grams									
3	560	3	185	185		185			185	
4	370	3	215	185		215			185	
2	390	2	185	185	185		215			185
18	710	8	125		95	125		150		150
19	640	10	150		105	105		150		165

In our experiments it was not decided whether the production of diastase in the pancreas was influenced by pancreatotoxin or not. But we can say now that the production or mobilization of diastase in the pancreas was not so much affected that the liver could not regulate the diastase content of the blood in the peripheral blood vessel, and also that the regulating action of the liver was not influenced by a dose of 3 or 4 cc. of this toxin.

Several experiments for control were undertaken with neurotoxin and the blood serum of normal rabbits. The results are shown in table 4.

TABLE 4

The diastatic activity of the peripheral blood before and after injection of the neurotoxin and the blood serum of normal rabbits

GUINEA PIG NUMBER	BODY WEIGHT	AMOUNT OF NEUROTOXIN OR NORMAL SERUM INJECTED INTO PERITONEAL CAVITY	DIASTATIC ACTIVITY $D_{38°}^{30'}$							
			Before injection	Day after injection of the neurotoxin or the blood serum of normal rabbits						
				3-4 (hours)	1	2	3	4	5	6
	grams									
33	510	3*	150	150	150					165
34	630	3*	150	150	150		165			150
35	580	3*	215	185	215	215				215
36	490	8*	165	125	105		125		165	
58	580	3†	150	125	150	125	125	125		
59	520	3†	125	125	125	150	125	125		

* Neurotoxin.

† Normal serum.

As we see in the above table, 3 to 4 cc. of the neurotoxin or the blood serum of normal rabbits caused no change in the diastase content in the blood, while the same dose of hepatotoxin markedly affected it.

Here we have also exceptions which may, however, be explained in the same manner as in the cases of pancreatotoxin. It can be now concluded that the hepatotoxin attacked the liver and affected its regulating power over the blood diastase.

The effect of the injection of adrenalin upon the blood diastase of guinea pigs, to which hepatotoxin was intraperitoneally injected. Starkenstein (1), Allen (12) and Watanabe (14) found no marked change in the

TABLE 5

The diastatic activity of the blood before and after injection of adrenalin or morphine

GUINEA PIGS WHICH WERE TREATED WITH HEPATOTOXIN	BODY WEIGHT	AMOUNT OF INJECTED ADRENALIN OR MORPHINE	DIASTATIC ACTIVITY	
			Before injection $D^{30'}_{38°}$	2 hours after injection $D^{30'}_{38°}$
number	*grams*			
11	430	Adrenalin, 0.1 mgm.	250	250
		Adrenalin, 0.2 mgm.	250	250
		Adrenalin, 0.4 mgm.	185	165
12	470	Adrenalin, 0.15 mgm.	185	185
		Adrenalin, 0.25 mgm.	215	215
		Adrenalin, 0.5 mgm.	165	150
3	560	Morphine, 0.02 gram	185	185
1	500	Morphine, 0.02 gram	250	250

diastase content of the blood after subcutaneous or intravenous injections of adrenalin or morphine. As we have proved in the foregoing experiments, the hepatotoxin can injure the liver, which regulates the blood diastase. Supposing, therefore, that adrenalin or morphine might affect the diastase content of the blood of the guinea pigs, which were treated with hepatotoxin, these drugs were subcutaneously injected in them (see table 5). But the diastase content of the blood was never affected.

The effect of Taka-diastase administered intraperitoneally upon the blood diastase. The injection of Taka-diastase might cause an increase in the diastatic activity of the blood of guinea pigs, especially after they

have been treated with hepatotoxin. In the following experiments a large dose of Taka-diastase was administered intraperitoneally, and the diastatic activity of the blood was examined (see table 6).

I was surprised to see such a marked decrease in the diastatic activity of the blood in spite of the injection of such a considerable quantity of diastatic substance.

In 1917 Richard Weil (14) proved that peptone markedly affects the liver. Taka-diastase contains peptone, besides diastatic ferment.

TABLE 6

The diastatic activity of the blood of guinea pigs, which were treated with hepatotoxin, before and after injection of Taka-diastase

GUINEA PIG NUMBER	BODY WEIGHT	AMOUNT OF TAKA-DIASTASE INJECTED INTO PERITONEAL CAVITY	DIASTATIC ACTIVITY $\left(D^{30'}_{38°}\right)$ AFTER INJECTION OF TAKA-DIASTASE									
			Before injection	Minutes				Day				
				15	30	60	180	1	2	3	4	5
	grams											
31*	500	0.05	125				95	85	125	125	150	
41*	860	0.05	150			105	105		125	125	125	150
44*	710	0.05	165			125	105		105	125	125	150
45*	730	0.5	125		125		85	75	105	105	150	
47*	710	0.05	150	105	95		95	85	95	105	105	125
15†	610	0.05	150				95	85	125	150	150	165
19†	640	0.05	165	150	125	125		105	125	125		
49‡	550	0.05	165			125	105		165	165		
50‡	550	0.05	150			125	105		165	185		

* Treated with hepatotoxin.

† Treated with pancreatotoxin.

‡ Not treated.

The latter may be eliminated by heating at 100°C. Hence, if Taka-diastase is heated at 100°C. there will remain the cocto-stable peptone. In the following experiments we have injected the heated Taka-diastase in normal guinea pigs in order to see if it affects the diastatic activity of the blood. Pure peptone dissolved in aqua destillata was also injected in guinea pigs intraperitoneally for control. The results are shown in table 7.

We see now from the table 7 that the heated Taka-diastase and peptone produced similarly a marked change in the blood diastase. The

curves of the diastase content in the blood following injections of hepatotoxin, Taka-diastase and peptone are almost the same. As such a decrease in the diastase content of the blood may be caused by 3 or 4 cc. of hepatotoxin, but not by the same dose of the other organ toxin, we can explain this decrease by assuming that the change in the blood diastase after injections of Taka-diastase and peptone was caused by the intoxication of the liver cells.

TABLE 7

The diastatic activity of the blood before and after injection of heated Taka-diastase or peptone

Guinea pig number	Body weight	Amount of Taka-diastase or peptone injected into peritoneal cavity	Diastatic activity $D\frac{30'}{38^\circ}$							
			Before injection	Day after injection of heated Taka-diastase or peptone						
				3-4 (hours)	1	2	3	4	5	6
	grams									
29	570	Heated Taka-diastase, 0.05	125	95	75	95	125	150		
30	520	Heated Taka-diastase, 0.05	125	105	95	105	125			
55	490	Peptone, 0.05	150	105	95	95	105	125	125	125
56	620	Peptone, 0.05	125	105	95	105	105	125	125	
57	610	Peptone, 0.05	150	105	105	105	125	125	150	

SUMMARY

1. The diastase content of the blood in the portal vein varies, while that in peripheral blood vessel remains constant.
2. Hepatotoxin administered intraperitoneally causes a marked decrease in the diastase content of the peripheral blood, while the same dose of pancreatotoxin or neurotoxin has no effect upon it.
3. Even the intraperitoneal injection of a large dose of Taka-diastase does not cause an increase in the blood diastase; on the contrary, it is followed by a marked decrease in the diastase content of the blood.
4. The diastatic activity of the blood is considerably weakened after the injection of heated Taka-diastase or peptone, which intoxicate the liver cells.
5. It seems sure that the diastase is regulated by the action of the liver cells.

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THE CHANGES IN THE CONTENT OF HEMOGLOBIN AND ERYTHROCYTES OF THE BLOOD IN MAN DURING SHORT EXPOSURES TO LOW OXYGEN

HAROLD W. GREGG, B. R. LUTZ AND EDWARD C. SCHNEIDER

From the Medical Research Laboratory of the Air Service, Mineola, New York

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The compensatory changes that occur in the blood of man and animals living under low oxygen tension have held the interest of many investigators. As long ago as 1878 Paul Bert (1) predicted that the blood of those living at high altitudes would be found to have a greater oxygen capacity than the blood of similar individuals living at lower levels, and he further suggested that the cause of the increase would be found to be the decrease in the partial pressure of the oxygen in the air respired. Since that time it has been clearly proved that a decrease in the partial pressure of oxygen of the respired air, regardless of the method of reduction, causes, if the reduction is great enough and the exposure continued through a sufficient interval of time, an increase in the erythrocytes and hemoglobin per unit volume of the blood. This increase has been found to be gradual, requiring from three to five and even more weeks to reach its maximal value (2), (3).

The time required for the increase in erythrocytes and hemoglobin to be first manifest has received some consideration. Campbell and Hoagland (4) carried rabbits to the summit of Pike's Peak, from an altitude of 6000 feet to one of 14,110, and found that in the ascent the number of red corpuscles had made an average increase of 9 per cent. Abderhalden (5), working with rabbits and rats, found an increase within a few hours. Ehrlich and Lazarus (6) state that the increase occurs immediately when considerable altitudes are reached. Douglas, Haldane, Henderson and Schneider (7) found in four men, several hours after their arrival on Pike's Peak, a slight increase in hemoglobin that varied for these individuals from 0.9 to 3.9 per cent. Schneider and Havens (2) were unable to demonstrate a clearly defined increase during the first seven hours spent on Pike's Peak, but within twenty-four hours there was a marked increase in the number of red

corpuscles and the percentage of hemoglobin. The increase occurred earliest and was most rapid in physically fit men. Dallwig, Kolls and Loevenhart (3) observed that animals kept at normal atmospheric pressure but under low oxygen showed a definite increase in the blood counts at the time of the first observations, viz., after two or three days. Six rabbits living at 352 mm. Hg. pressure required as much as twenty-four to forty-eight hours for the increase to become definite.

All of the above observations were made after hours or even days of exposure to the effects of high altitudes and deficiency of oxygen. In order that the aviator may benefit by blood compensatory changes they would have to occur during exposures of thirty minutes to three hours. We have, therefore, investigated the blood changes that occur in men subjected to a lowered barometric pressure in a low pressure chamber and to low oxygen, 10 per cent, for intervals not exceeding two hours.

In a preliminary report by one of us (8) it was shown that in short exposures at least 25 per cent of all men examined had a well-defined increase in the percentage of hemoglobin. Corbett and Bazett (9) using a low oxygen method conclude that after about half an hour some degree of blood concentration may occur.

Blood for the estimation of hemoglobin was obtained in the usual manner by pricking the finger. In a few experiments with the Dreyer Nitrogen Apparatus it was taken from the lobe of the ear. In many cases the blood from the finger was compared with blood taken without stasis from a prominent vein in the forearm. Blood was obtained from the vein with a 10 cc. Record syringe and put in a short tube containing a little sodium oxalate. After a thorough stirring a few drops were taken with a pipette and put on a watch glass from which the sample was immediately taken into the blood pipette.

In the earlier experiments the Gower-Haldane carbon monoxide method (10) was used. Each sample was matched at once with the standard in the low pressure chamber by the aid of a white background and a "daylight" electric lamp. It was found more convenient, because of difficulty with the carbon monoxide supply, to dilute the blood in small test tubes containing 0.4 per cent ammonia. These samples were later transferred with proper rinsing to the Gower-Haldane graduated tube, saturated with carbon monoxide, and diluted further. The last sample taken in the experiment was always received directly in the Gower-Haldane tube.

Several experiments were carried out using the Palmer method (11). It was found difficult to keep the 1 per cent standard carbon monoxide blood, therefore the normal samples were usually considered as 100 per cent and the later samples were matched against them with the Duboscq colorimeter. The most convenient method was found to be a modification of the Palmer method which consisted in diluting the blood in 5 cc. of N/10 hydrochloric acid, instead of 0.4 per cent ammonia. The diluting fluid was measured in small test tubes which were stoppered with cotton and taken into the low pressure chamber. The blood was rinsed immediately into the diluting fluid from the 0.05 cc. pipette. The samples were matched at the end of the experiment as in the Palmer method.

Blood for the erythrocyte counts was taken by pricking the finger so that a free flow was obtained. The same Thoma mixing pipette was used for all comparative counts, and the blood was diluted in Hayem's solution. Two drops were put in a Levy double counting chamber with Neubauer ruling.

Experiments in the low pressure chamber. These experiments constitute the major part of this study. Throughout the work we adopted the plan of lowering the barometric pressure within the chamber at a rate that would be comparable to ascending in the air at the rate of 1000 feet per minute. The following altitudes were employed: 425, 395 and 380 mm. Hg., which are the pressures ordinarily encountered at 15,000, 17,000 and 18,000 feet respectively. The desired pressure having been attained it was then maintained for a time during which several samples of blood were taken and other observations made. From 30 to 100 minutes was the usual exposure to the lowered pressure.

A total of forty-five experiments was made upon thirty-five men, five of whom served as subjects two to five times. In fifteen of the experiments the hemoglobin determinations were made on blood from a vein of the arm as well as from the peripheral vessels of the finger. Also in fifteen other experiments the erythrocytes were counted as a check against the final hemoglobin determination. The data from these fifteen cases are given in table 2. The results for the thirty examinations in which the hemoglobin changes only were considered are collected in table 1.

The blood changes were definite in thirty-five, or in 78 per cent, of the experiments. In only ten, or 22 per cent, was the period of exposure to the lowered barometric pressure too brief or not sufficiently low to cause the blood response. Of the eight cases held at a pressure

TABLE 1

Low barometric pressure and hemoglobin

NAME AND DATE	MINUTES	HEMOGLOBIN	INCREASE IN PER CENT
15,000 feet			
A. F. H. May 21	0	94	
	15	94	
	55	97	
	85	98	4.3
G. W. D. May 23	0	104	
	15	104	
	58	105	
	75	106	1.9
W. H. G. May 24	0	96	
	15	103	
	25	106	
	35	106	
	45	105	
	65	105	9.4
W. B. M. May 27	0	104	
	15	102	
	25	104	
	35	104	
	45	104	
	55	104	
	75	110	5.8
W. O. K. June 6, 1918	0	106	
	25	106	
	45	106	
	65	107	
	83	107	0.0
	0-V	106	
	93-V	107	0.0
17,000 feet			
F. S. V. June 26, 1918	0	92	
	25	93	
	40	94	
	54	94	2.2
	0-V	98	
	56-V	100	2.0
18,000 feet			
N. E. F. June 7, 1918	0	94	
	25	96	
	45	96	
	65	97	
	83	100	6.9
	0-V	95	
	85-V	99	4.2
R. S. S. June 10, 1918	0	102	
	25	104	
	35	104	
	50	106	
	63	107	
	71	106	3.9
	0-V	102	
	75-V	104	2.0
N. G. B. June 11, 1918	0	107	
	25	110	
	35	110	
	50	110	
	65	112	
	80	112	
	95	111	
	100	112	4.7
	0-V	107	
	103-V	111	3.7
W. B. M. June 12, 1918	0	105	
	27	105	
	50	111	
	55	110	4.8
	0-V	105	
	56-V	109	3.8
H. M. T. June 14, 1918	0	112	
	25	112	
	43	114	
	60	114	
	79	114	1.8
	0-V	108	
	75-V	110	1.8

TABLE 1—*Continued*

NAME AND DATE	MINUTES	HEMO-GLOBIN	INCREASE IN PER CENT	NAME AND DATE	MINUTES	HEMO-GLOBIN	INCREASE IN PER CENT
B. M. L.	0	96		W. C. W.	0	98	
June 17,	26	96		July 16,	64	102	
1918	40	99		1918	78	104	6.1
	56	106					
	75	104	8.3	L. G. R.	0	100	
				July 19,	40	100	
A. W. L.	0	99		1918	68	102	
June 18,	26	100			80	103	3.0
1918	43	98					
	55	100		L. J. S.	0	108	
	65	100		July 26,	45	108	
	75	98		1918	63	108	0.0
	85	100	0.0				
	0-V	98		E. W. B.	0	98	
	86-V	100	2.3	June 30,	25	98	
				1918	40	98	
L. F. M.	0	103			60	100	
July 21,	25	102			80	102	
1918	35	102			92	104	6.1
	45	104					
	55	107		I. M.	0	108	
	65	106	3.0	July 1,	79	111	2.8
	0-V	104		1918	0-V	107	
	68-V	106	1.9		80-V	110	2.8
G. C. W.	0	98		H. W. B.	0	106	
June 25,	27	94		July 2,	58	110	3.8
1918	41	94		1918	0-V	108	
	50	97			60-V	112	3.7
	82	100	2.0				
	0-V	97		B. F.	0	106	
	83-V	99	2.1	July 2,	50	106	
				1918	66	109	2.7
W. B. M.	0	104					
June 28,	25	103		D. T. R.	0	98	
1918	40	104		July 2,	40	97	
	55	107		1918	60	97	
	72	110	5.8		88	97	0.0
	0-V	106			0-V	100	
	75-V	108	1.9		89-V	100	0.0
A. F. H.	0	94		H. J. M.	0	92	
July 15,	40	95		July 5,	41	89	
1918	60	95		1918	59	90	0.0
	78	98	4.3		0-V	90	
					61-V	89	0.0

TABLE 1—*Concluded*

NAME AND DATE	MINUTES	HEMO-GLOBIN	INCREASE IN PER CENT	NAME AND DATE	MINUTES	HEMO-GLOBIN	INCREASE IN PER CENT
A. F. H.	0	99		E. C. S.	0	100	
July 8,	40	100		April 29,	29	108	
1918	60	104		1919	57	107	
	86	104	5.0		77	109	
					92	107	7.0
P. S. B.	0	104					
July 9,	36	102		N. E. B.	0	100	
1918	60	110		1919	42	108	
	85	109	4.8		75	110	
					76	110	10.0

V = Blood from vein.

corresponding to 15,000 feet three, or 38 per cent, failed to compensate, while among the five at 17,000 feet one, or 20 per cent, and among the thirty-two at 18,000 feet six, or 19 per cent, did not respond.

There were six men, 13 per cent, who showed a well-defined increase in the hemoglobin within the first 26 minutes of the experiment. This included the time allowed for ascent which varied from 15 to 18 minutes. The majority of men require between 45 and 60 minutes for the increase to be sufficient to be detected by the methods used.

There was a marked parallelism between the blood from the finger and blood from the vein, but in almost every case that from the vein was found to contain slightly less hemoglobin than that from the finger.

In the fifteen experiments in which the erythrocytes were counted there was an increase per cubic millimeter in each case in which the hemoglobin gave evidence of concentration. In five of these experiments no blood change occurred. The relation between the increase in the number of erythrocytes and hemoglobin shows a greater increase in erythrocytes than hemoglobin in seven of the ten positive cases.

W. B. M. served five times as a subject, once at 425 and four times at 380 mm. Hg. His hemoglobin percentage increases were 5.8, 4.8, 5.8, 0 and 8.8 respectively. A. F. H. was examined four times, one at 425 and three at 380 mm. respectively, with 4.3, 5, 4.3 and 0 per cent increases in hemoglobin. W. H. G. in two times showed 9.4 and 6.1 per cent, and B. R. L. in two experiments showed 8.4 and 8 per cent rises in hemoglobin. W. O. K. at 425 mm. failed to show a response, but at 395 mm. Hg. had an increase of 7 per cent in hemoglobin. The surprising feature of these repeated cases is the fact that, when a defi-

TABLE 2

Erythrocytes and hemoglobin in low pressure chamber

NAME	DATE	PRESSURE	ALTITUDE	LENGTH OF EXPERIMENT	HEMOGLOBIN			ERYTHROCYTES		
					Normal	At end of hold	Change	Normal	At end of hold	Change
		mm.	*feet*	*minutes*			*per cent*			*per cent*
F. C. P.	December 9, 1918	425	15,000	75	100	98	0.0	5,048,000	5,256,000	0.0
T. S. G.	December 10, 1918	425	15,000	60	100	104	4.0	5,120,000	5,368,000	4.8
C. P. C.	December 13, 1918	425	15,000	61	122	120	0.0	4,792,000	4,480,000	0.0
J. H. N.	December 31, 1918	395	17,000	91	83	91	9.8	4,440,000	5,064,000	12.3
K. O. W.	January 6, 1919	395	17,000	82	82	89	8.3	4,308,000	4,966,000	15.2
F. D.	January 13, 1919	395	17,000	86	91	91	0.0	5,200,000	4,983,000	0.0
W. O. K.	January 14, 1919	395	17,000	102	86	92	7.0	4,868,000	5,375,000	10.4
W. H. G.	July 10, 1918	380	18,000	102	98	104	6.1	5,216,000	5,720,000	9.5
E. A. R.	July 13, 1918	380	18,000	83	95	98	3.2	5,232,000	6,080,000	16.0
A. F. H.	July 30, 1918	380	18,000	69	99	100	0.0	5,080,000	5,012,000	0.0
W. B. M.	July 31, 1918	380	18,000	56	104	103	0.0	4,936,000	4,704,000	0.0
B. R. L.	August 5, 1918	380	18,000	145	107	116	8.4	4,672,000	5,600,000	20.0
W. B. M.	December 27, 1918	380	18,000	85	91	99	8.8	4,752,000	4,931,000	3.8
K. O. N.	April 30, 1919	380	18,000	83	100	106	6.0	4,774,000	5,520,000	13.7
B. R. L.	May 12, 1919	380	18,000	80	100	108	8.0	5,274,000	5,732,000	8.1

The ascent was made at the rate of 1000 feet a minute.

nite response does occur, the total increase is so often approximately the same in an individual. Just why there was failure in the blood response in the two men who ordinarily reacted well to low oxygen is not indicated in our data. It is evident that there are physiological conditions under which an individual may not react in equal degree every time he encounters a given barometric pressure. We believe that when the blood fails to give the increase in red corpuscles and hemoglobin under the low oxygen of low barometric pressures that a heavier burden is thrown upon the respiration and the circulation of the blood.

Experiments at normal atmospheric pressure and 10 per cent oxygen. In these experiments the subject breathed atmospheric air diluted with nitrogen by the Dreyer Nitrogen Low Oxygen apparatus (12). Starting with undiluted air, 20.96 per cent oxygen, the subject breathing through a mask, the nitrogen was gradually added in greater and greater proportion so that by the end of 20 minutes the mixture contained only 10 per cent oxygen. Ten per cent oxygen at 760 mm. Hg. pressure corresponds in oxygen partial pressure to an altitude of approximately 19,000 feet. The subject of the experiment was held at this level of oxygen for from 30 to 90 minutes, thus he was kept under low oxygen for a period of from 50 to as much as 112 minutes.

Only seven men were examined for the hemoglobin changes by this method, four of them gave a positive response (see table 3). In three of the men the increase in hemoglobin had already begun when the first sample of blood was taken, 20 to 26 minutes, which was soon after 10 per cent oxygen was reached.

Two of the men, W. O. K. and E. A. R., were also tested in the low pressure chamber. W. O. K. showed an 8 per cent increase in hemoglobin under 10 per cent oxygen and 7 per cent under 395 mm. barometric pressure. E. A. R. with the low oxygen gave 5 per cent increase in hemoglobin and with the low pressure 380 mm. Hg., gave 3.2 per cent.

The results obtained by the two methods of subjecting men to low oxygen—lowered barometric pressure and lowered oxygen percentage—show that an increase in hemoglobin and the red corpuscles of the blood may result during short exposures. About 60 per cent of the men subjected to 10 per cent oxygen and 78 per cent of those subjected to low barometric pressure showed within from 15 to 90 minutes clearly defined increases in hemoglobin that ranged between 1.8 and 10 per cent. From the data presented it appears that the majority of men make the blood compensation rather quickly. Some delay is usually present in this response to low oxygen, but the lag is surprisingly short.

We have not investigated the mechanism by means of which these quick and early blood changes occur, when the organism is subjected to lowered partial pressure of oxygen. Views have differed as to the mechanism by which the marked increases in erythrocytes and hemoglobin occur during residence at a high altitude. Schneider and Havens (2) have given their opinion of the changes in the blood on adaptation to high altitudes as follows:

TABLE 3

Normal atmospheric pressure, oxygen 10 per cent

NAME AND DATE	MINUTES	HAEMOGLOBIN	INCREASE IN PER CENT	NAME AND DATE	MINUTES	HEMOGLOBIN	INCREASE IN PER CENT
P. A.	0	98		C. A. C.	0	115	
May 25,	20	101		June 1,	22	112	
1918	37	99		1918	40	113	
	57	99	0.0		55	116	
					65	114	
G. B. H.	0	100			75	114	
May 28,	18	100			90	116	
1918	34	100			100	113	0.0
	46	100					
	70	100		W. O. K.	0	100	
	82	104	4.0	June 3,	26	102	
				1918	39	106	
C. N.	0	109			50	108	8.0
May 29,	20	107					
1918	40	107		E. A. R.	0	100	
	75	106		June 4,	20	102	
	104	106		1918	40	103	
	112	106	0.0		55	103	
					74	105	
W. A. B.	0	104			81	105	5.0
May 31,	22	106			0-V	90	
1918	41	106			80-V	102	13.3
	56	108					
	71	112					
	81	112					
	91	112	7.7				

V = Blood from vein.

A rapid increase in the number of red corpuscles and percentage of hemoglobin in the blood of the peripheral vessels occurs during the first two to four days of residence at the high altitude, then follows a more gradual increase for

about three weeks. The initial rapid increase is brought about in part by throwing into the systemic circulation a large number of red corpuscles that under ordinary circumstances at low altitudes are side-tracked and inactive, and in part by a concentration resulting from a loss of fluid in the blood. The more gradual increase in red corpuscles and hemoglobin extending over several weeks is brought about by the increased activity of the blood-forming centers so that there results a large increase in the total number of corpuscles and amount of hemoglobin.

The question which presents itself here is whether the early increase in hemoglobin and red corpuscles, such as we obtained within the short space of an hour, is to be attributed to concentration of the blood, i.e., a reduction in the total blood volume, or to changes in the distribution of the erythrocytes. A suddenly increased production of hemoglobin and erythrocytes by the bone marrow is improbable. That the increase is not caused by an increased evaporation of water from the body is indicated by the conditions of experimentation and the fact that in some men the change is already well developed within a 15 to 20 minute period and that perspiration is not noticeably increased. It has been claimed that all aviators engaged on long patrols at 17,000 feet, or over, complain of over-filling of the bladder. Birley (13) looks upon this as confirmatory of the theory that one factor in the reaction of the organism to lowered barometric pressure is a concentration of the blood at the expense of the plasma. Against this theory of a polyuria we urge that the time, 15 to 20 minutes as seen in a few cases, is too short. If this were the mechanism, all subjects should be conscious of the filling of the bladder. The majority of our subjects have not been conscious of an increased action of the kidneys. In fact we are inclined to believe that only the nervousness experienced during the first time or so spent in the low pressure chamber gives rise to the sensation of an increased sensation of urine. Several observations made, in and out of the low pressure chamber in this laboratory, on the secretion of urine fail to confirm the polyuria theory.

The increased production of urine during flights at high altitudes finds an explanation in the action of cold. Against the likelihood of this increased urine formation being evidenced in blood concentration we have the studies of Bogert, Underhill and Mendel (15) in which they introduced large volumes of fluids without appreciably affecting the unit blood content of hemoglobin.

Another possibility is that lowered oxygen tension changes the property of the muscles so that they absorb a larger volume of water and

in sufficient quantity to reduce the blood volume. We know of no experimental proof for this view.

The percentage of increase in hemoglobin and erythrocytes observed in these short exposures to low oxygen is within the limits of those observed after various forms of physical exertion. Schneider and Havens (14) found that exercise increased the hemoglobin to from 3.5 to 11 per cent and the number of red corpuscles per cubic millimeter to from 3.2 to 22 per cent. They held that this increase was the result of throwing into the systemic circulation a large number of erythrocytes that under ordinary circumstances are side-tracked and inactive. The same explanation might be advanced for the low oxygen compensatory blood changes. A decision as to the value of the concentration theory and the theory of the dormant supply of erythrocytes cannot be made at this time.

The physiological significance of an increase in erythrocytes and hemoglobin during exposure to low oxygen is that a unit volume of blood can carry for a given oxygen pressure more oxygen than normally. The supposition is that the aviator whose blood concentrates will, other things being equal, tolerate high altitudes more comfortably and more efficiently than the man who does not react with an increase in erythrocytes and hemoglobin.

SUMMARY

1. Low oxygen tension was produced by lowering the barometric pressure in different experiments to 380, 395 and 425 mm. Hg., and by replacing oxygen by nitrogen gradually until 10 per cent oxygen was reached. The subjects were maintained at the low oxygen tensions from periods varying from 30 minutes to 145 minutes.

2. Blood for the estimation of hemoglobin was taken from a finger and a vein. The determinations were made by the Gower-Haldane carbon monoxide method, by the Palmer method, and by a modified Palmer method using hydrochloric acid.

3. An increase in hemoglobin was obtained under reduced barometric pressure in 78 per cent of all examinations made. The majority of the men required between 40 and 60 minutes for the increase to become definite, 13 per cent showed a well defined increase within 26 minutes. In the experiments with 10 per cent oxygen 57 per cent gave the increase in hemoglobin.

4. In fifteen cases in which the erythrocytes and hemoglobin were determined corresponding changes occurred in both, 66 per cent were positive. The erythrocyte increase ranged between 3.8 and 20 per cent, the hemoglobin between 3.2 and 9.8 per cent.

5. In the several experiments on the same individual, the increase in the hemoglobin was approximately the same each time.

6. The blood concentration theory and the theory of the dormant supply of erythrocytes are briefly contrasted.

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CIRCULATORY RESPONSES TO LOW OXYGEN TENSIONS

BRENTON R. LUTZ AND EDWARD C. SCHNEIDER

From the Medical Research Laboratory of the Air Service, Mineola, New York

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It is a well established fact that residence at high altitudes exerts a profound influence on the human body. Because of the recent development of aviation, which has made rapid ascents to very high altitudes possible, the knowledge of the effects of short exposures to the influence of altitude assumes practical importance. The adaptive reactions to altitude observed in men who take up residence at a high altitude develop rather slowly and are of a fairly permanent character (1). The aviator does not remain long enough at a high altitude to benefit from slow adaptive physiological changes. If he tolerates and does well, he must depend upon rapid compensatory changes to provide the oxygen needed by the tissues. That the body is capable of responding to abrupt and great changes in atmospheric pressure has been proved by studies made in this laboratory (2). Among the physiological responses made to low oxygen tensions are those of the circulatory mechanism. The object of this paper is to present observations on the pulse rate and the arterial blood pressures made upon men who were subjected to low oxygen tension produced in three ways, by low barometric pressures in a low pressure chamber, by low percentage of oxygen caused by rebreathing under normal atmospheric pressure, and by diluting the respired air with increasing amounts of nitrogen.

The low pressure chamber and its control has been briefly described elsewhere (3). The rebreathing experiments were made with the Henderson-Pierce rebreathing machine (3). The Dreyer method (4) was used to dilute atmospheric air with nitrogen which was delivered to the subject by means of an American model of a Tissot gas mask.

Two types of experiments have been carried on. In one the oxygen tension was gradually reduced until the mental condition of the subject showed that he was no longer able to compensate, or until syncope appeared. In the other group of experiments the oxygen tension

was reduced at a rate corresponding to an ascent of 1000 feet per minute until a desired level,—that is, 15,000, 17,000, 18,000 feet,—was reached, after which the level was maintained for from 30 to 90 or more minutes.

Throughout all experiments the subjects were seated, and it was the rule to count the pulse rate for half a minute during each minute of the experiment. Usually the count was begun at 45 on the seconds dial of a stop-watch and continued to 15 and then recorded as though taken on the minute. In the remaining portion of the minute the blood pressures were taken. To keep such a record requires close attention and extensive experience. During each experiment one man was held responsible for all these determinations and was relieved of the necessity of watching the condition of the subject and arrangements of experimentation.

The arterial blood pressures were determined by the auscultatory method with the aid of a Tycos sphygmomanometer which was adjusted over the brachial artery of the left arm. A Bowles stethoscope with special arm band was used. The systolic pressure was read at the beginning of the first phase and the diastolic pressure was measured at the fourth phase, that is, at the dulling of the intense sounds of the third phase. In many of the low pressure chamber experiments, in which the pump was run continuously, the diastolic determinations had to be omitted because of noise.

In the selection of subjects an effort was made to secure men who had not been doing physical work during the hour previous to the experiment. Before any observations were made the subject was allowed to sit quietly for a while, after which a number of preliminary determinations of the pulse rate and arterial blood pressures were made to establish the so-called normal. Occasionally the first time a subject appeared for the low oxygen test he showed some degree of anxiety or excitement in a slightly rapid pulse or increased systolic pressure. After a few moments of tactful conversation this nervousness was usually overcome. In much of our work we have used men accustomed to being subjects, and in these the excitement effect is often absent, or if present almost negligible. In all experiments in which an attempt is made to detect the earliest effects of low oxygen, this psychic factor has to be considered. Unquestionably it often masks the onset of heart rate acceleration due to low oxygen.

Earlier work on heart rate during exposures to low oxygen tension. When the oxygen tension of the respired air is decreased, the blood for

a time may be less completely saturated with oxygen than when air of normal composition and pressure is breathed. During such a condition the tissues would very likely be inadequately supplied with oxygen. If during this period the blood contains less oxygen than normally, and the rate of blood flow through the capillaries is increased, the tissues will be provided with the oxygen demanded for their activity. More blood flowing to the tissues, even though it contains a lessened amount of oxygen, results to some extent in maintaining the oxygen tension in the tissues.

Throughout our experimental work with low oxygen we have assumed that an increase in the rate of the heart beat, the arterial pressures being maintained within normal limits, meant an increase in the per-minute output of the heart. Under ordinary circumstances an increase in the pulse rate during exercise is recognized as satisfactory evidence that the output of the heart has increased and the flow of the blood has accelerated.

The idea that an increase in the heart rate is a method of compensating for lack of oxygen is by no means new. Finkler (5) in 1875 induced anemia in dogs by bleeding and found that the decrease in the oxygen content of the blood may stimulate both the heart and respiration to greater activity. These, as Lusk (6) has pointed out, are efforts of compensation for the decrease in oxygen, although nothing resembling asphyxia is present. Kohler (7) in 1877 interfered with the respiration of rabbits by compressing the trachea by means of a lead wire tied around it. This was followed by compensation by means of increased respiration and heart activity so that there was no lack of oxygen in the animals. In these animals the heart hypertrophied.

Experimental studies on men living at a high altitude have seemed to prove an increased rate of blood flow. Schneider and Sisco (8), on Pike's Peak by use of Stewart's hand-colorimeters, concluded that "the rate of blood flow in the hands of six men examined was increased . . . . by an amount varying from 30 to 70 per cent." The increase in the rate of flow has been associated in part with an augmented rate of heart beat and a fall in the venous pressure, also in part with a dilatation of the arterioles. Kuhn (9) working on Monte Rosa demonstrated by calculations made from determinations of the oxygen capacity of the blood, the total oxygen consumption, and the pulse rate, that the heart rate responds to the influence of lowered barometric pressure by increasing its output per minute.

Hasselbach and Lindhard (10) working with three men in a pneumatic chamber failed to prove an increased blood flow with the nitrous-oxide method. It should be noted, however, that their pressure changes were too small to produce profound change. They maintained pressures of 589 to 514 mm. Hg. (6800 to 10,400 feet) for five to seven days and attained these pressures very slowly. Under these conditions other compensatory changes might have been sufficient to meet the call for oxygen.

Our knowledge of the influence of high altitudes on circulation has been secured chiefly from men living at high altitudes on mountains. Of all the circulatory changes due to diminished barometric pressure, the acceleration of the heart rate has been most studied. Mountain ascents, even when made passively by railway car or automobile are slow, 8000 feet in an hour and a half or longer, when compared with altitude flights in an aeroplane. It has been shown by studies on Pike's Peak (11), (14,110 feet), that the pulse rate does not accelerate immediately on arrival at the summit. It accelerates gradually in those who ascend passively by train and remain well, and requires several days to reach the maximum rates. In men who become mountain or altitude-sick the augmentation comes on earlier and is greater than in those who remain well. Later the rate returns to the normal for the particular altitude. In men fatigued by walking to the summit the high altitude heart rate is usually established within a few hours.

Changes in heart rate during a gradual decrease in oxygen tension. Seventeen men served as subjects for a series of examinations in which the action of a steadily decreasing barometric pressure was compared with that of a steady decrease in the oxygen percentage by the rebreathing method. In this work it was customary to give the rebreathing test first, and on a later day the low pressure test. From the rebreathing data, in which the final oxygen percentage and the duration of the test in minutes were recorded, we calculated the barometric pressure that was equivalent in oxygen tension to the final rebreathing oxygen per cent, and then determined the rate at which the pressure should be lowered in the low pressure chamber. It was thus possible to reproduce with a fair degree of accuracy in the low pressure chamber test the oxygen tension changes experienced during rebreathing examination. In two pairs of experiments (see G. F. H. and F. D., table 1) the rebreathing test was prolonged by introducing into the tank of the apparatus a continuous flow of oxygen. This, in effect, made the altitude ascent about three times slower than that of the average test. In about 70 per cent of the tests the men were carried down in oxygen

until they became inefficient as judged by the psychologist or the failure of the compensatory mechanism.

The response of the heart during rebreathing tests of 25 to 30 minutes duration has been described by Schneider (12). He reported that in men under a gradually decreasing oxygen supply the heart rate soon began to accelerate, at first by a slight increase of from one to three beats, and later by a very marked acceleration when the oxygen had fallen to between 13 and 9 per cent. The heart rate was shown to accelerate in a few men as early as 17.5 per cent of oxygen (5000 feet) while 12 per cent of all cases examined began to respond between 15.5 and 14.9 per cent oxygen, (8000 to 9000 feet).

In our series of low pressure and low oxygen percentage comparisons the similarity of the circulatory responses made by the individual to the two conditions was most striking. The data have been analyzed and tabulated in table 1. It was impossible to have the subject in exactly the same condition for each test because the two types of tests were separated by at least 24 hours and in one case by 18 days. Furthermore the degree of apprehension in the subjects differed for the tests. Some men dreaded going into the low pressure chamber and others disliked the mouthpiece and nose clip of the rebreather. The apprehension was of course registered in a quickened pulse rate or increased systolic pressure when the normals of each were compared with the determinations made at the start of the actual experiment.

When the psychic factor was not in evidence, the pulse rate, in each of the two kinds of low oxygen experiments, maintained for a short time the normal or pre-experiment rate and then gradually began to accelerate. In the majority of cases there was at first a slow increase in rate, but when the oxygen tension had fallen to that corresponding to from 15 to 10 per cent oxygen at normal atmospheric pressure, it gave way to a more rapid rate of acceleration.

The beginning of a pulse rate acceleration has been determined as to time and pressure, or oxygen per cent. Three of the men showed in both the low pressure chamber and under low oxygen per cent a definite gradual acceleration which began with the first change in pressure or oxygen per cent at the beginning of the experiment. In the seventeen comparisons the latest onset in the acceleration occurred at 15.2 per cent oxygen (8400 feet). We believe that the evidence proves the heart to be responsive to a slight decrease in oxygen in the air respired if the psychic acceleration is satisfactorily eliminated, as it has been in a great many of our cases. Fourteen times in this series of

comparisons, or in 41 per cent of the tests, the acceleration began between 17 and 15 per cent oxygen, that is, between altitudes of 5800 and 8800 feet. All men in this series showed an increase in heart rate before an oxygen percentage corresponding to 9000 feet was reached. Omitting from our calculations the cases that gave an immediate acceleration there were nine cases, or 26 per cent, that responded with an increase in heart rate at 18 or more per cent of oxygen (4000 feet or less).

It is generally found that men living at moderately high altitudes, 6000 to 9500 feet, to which they are acclimated, do not show an augmentation in the rate of heart beat. The reaction shown in our tests is an immediate compensation to low oxygen, and as will be seen later is not necessarily a permanent change which would be maintained so long as the particular oxygen tension was held.

The normal pulse rates and the maximum rates, which occurred when the oxygen tension was lowest, are recorded in table 1. In several of the runs the final heart rate was only 16 or 18 beats per minute above the preliminary or normal rate. In other men the acceleration was 45 and in one case 57 beats per minute. The smaller acceleration usually occurred in men who reached only 9 or 10 per cent oxygen, while the greater increase occurred with 6 and 7 per cent oxygen. The percentage of acceleration brings out more clearly the comparative differences; thus R. M. B. at 10 per cent oxygen had an acceleration of 19 per cent, and G. F. H. at 6.3 per cent oxygen showed a 79.3 per cent increase.

Those who would account for the circulatory change at reduced atmospheric pressure apart from decreased oxygen tension, would find it difficult to explain the parallelism observed in the responses of the heart to the two methods of experimentation employed in these pairs of tests, under low barometric pressure and low oxygen caused by rebreathing at normal atmospheric pressure. The heart rate responded at so nearly the same percentage of oxygen in eleven of the seventeen pairs of experiments that we are justified in speaking of them as duplicate responses. In F. D. the acceleration began at 17 and 16.9 per cent, in D. T. R. at 15.5 and 15.8 per cent, in R. M. B. at 15.4 and 16 per cent, in C. N. at 15.7 and 15.5 per cent and in G. M. at 19.2 and 20 per cent for the low pressure chamber and the rebreathing experiments respectively. The plotted curves (see fig. 1) showing the relationship between low pressure and low oxygen changes likewise indicate that the same cause must be operating in the two methods of experimentation.

TABLE 1

Comparison of rebreathing and low pressure chamber experiments. Oxygen tension reduced gradually until the end

NAME	DATE	REBREATHING OR L. P. CHAMBER	LENGTH IN MINUTES	FINAL OXYGEN		PULSE			BEGINNING OF ACCELERATION		
				Per cent or barometer	Tension	Begin	End	Per cent increase	Minute	Per cent or barometer	Tension O_2
G. F. H.	3/ 4/18	Rebr.	85	8.5	60.8	73	118	61.6	12	19	145
	3/ 6/18	L. P. C.	85	330	69.1	66	98	48.5	31	600	126
G. F. H.	3/ 5/18	Rebr.	24	6.3	47.9	72	129	79.3	8	16.3	124
	3/ 8/18	L. P. C.	25	325	67.1	69	111	60.9	2	690	145
S. I.	3/ 4/18	Rebr.	31	9.3	70.6	82	111	35.4	At once		
	3/ 8/18	L. P. C.	35	338	70.8	86	120	39.5	At once		
F. D.	3/ 5/18	Rebr.	36	7.3	55.5	84	126	50.0	12	17	129
	3/ 4/18	L. P. C.	32	365	76.5	70	108	54.4	4	610	128
F. D.	3/ 6/18	Rebr.	90	8.0	60.8	80	117	46.2	19	18	137
	3/ 7/18	L. P. C.	90	310	64.9	84	122	45.2	40	550	115
C. H.	4/ 9/18	Rebr.	26.5	7.7	58.5	92	123	33.7	At once		
	4/12/18	L. P. C.	27	282	59.0	84	112	33.3	9	610	128
C. K. R.	4/ 9/18	Rebr.	26.5	8.2	62.4	88	123	39.8	At once		
	4/13/18	L P C.	27.0	295	61.8	96	123	28.1	At once		
D. T. R.	4/20/18	Rebr.	27.5	7.8	59.4	81	114	40.7	12	15.5	118
	4/24/18	L. P. C.	27.0	308	64.5	81	114	40.7	12	570	119

R. B.	4/ 4/18	Rebr.	25.5	8.6	65.4	78	123	57.7	At once		
	4/22/18	L. P. C.	27.0	310	64.9	74	112	51.4	At once		
G. W. D.	4/10/18	Rebr.	26.5	6.9	52.5	80	114	42.5	At once		
	4/15/18	L. P. C.	25	300	62.8	87	105	20.7	At once		
I. M.	4/10/18	Rebr.	25	9.7	73.6	64	80	25.0	6	18.3	139
	4/17/18	L. P. C.	25	360	75.5	64	92	43.8	At once		
R. M. B	4/17/18	Rebr.	25	10.0	76.0	84	100	19.0	11	16.0	122
	4/19/18	L. P. C.	25	360	75.5	78	99	26.9	12	560	117
A M. J.	4/30/18	Rebr.	25	9.2	70.0	90	108	20.0	10	16.3	124
	5/ 1/18	L. P. C.	25	325	67.1	84	108	28.5	3	709	148
F. L. D.	4/12/18	Rebr.	20	9.3	70.6	64	90	40.5	14	18.0	137
	4/16/18	L. P. C.	20	335	70.2	72	90	25.0	8	590	124
C. N.	4/15/18	Rebr.	22	9.8	74.5	64	86	34.4	11	15.5	118
	4/25/18	L. P. C.	22	345	72.2	58	76	31.1	11	565	117
L. S. L.	4/11/18	Rebr.	28	9.5	72.2	60	82	36.7	6	18.5	141
	4/13/18	L. P. C.	28	350	73.4	59	84	42.4	At once		
G. M.	4/23/18	Rebr.	25	10.0	76.0	66	90	36.4	2	20.0	152
	4/29/18	L. P. C.	25	360	75.5	66	90	36.4	4	696	146

The difference between the normal and maximum rates expressed in percentage increase also shows that the pulse response took place in about equal degree when equal oxygen tensions were reached. The discrepancies for G. F. H., 1 and 2, and G. W. D. in table 1 are explained by failure to reach the same lower limit in each experiment. The wide difference shown by I. M. and F. L. D. cannot be explained by our data. The parallelism in response is best shown by C. H., 33.7 and 33.3 per cent, C. N., 31.1 and 34.4 per cent, and G. M. with a 36.4 per cent total acceleration in the low pressure and low oxygen by the rebreathing method. The difficulty in reproducing exactly conditions

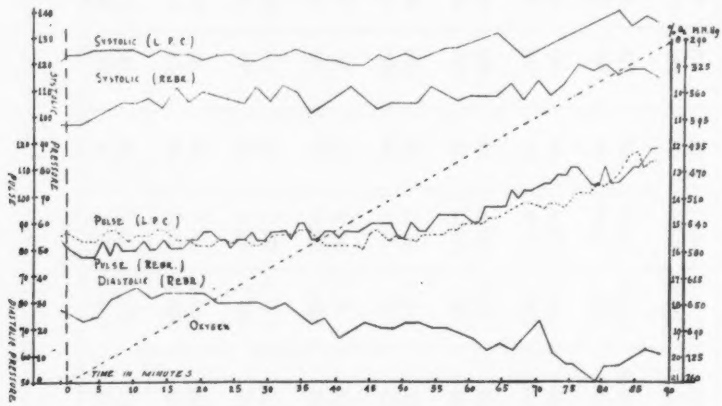

Fig. 1. F. D. Comparison of the rebreathing and low pressure chamber methods. Blood pressures and pulse rate taken every minute. This case illustrates the close correspondence in pulse response, although the experiments were made on different days. See table 1.

in experimentation and securing subjects who are exactly the same physically on two different days makes the parallelism here reported all the more striking. It is evidence for the theory that lack of oxygen, or decrease in oxygen tension, is the cause of the heart rate response under the two very different procedures.

Two sets of comparative experiments were conducted on G. F. H., and F. D. (see table 1), one of moderate length for each, and one extending over 85 and 90 minutes. In each set the oxygen and the pressure were gradually decreased throughout the period of experimentation. Rebreathing experiments carried on in this laboratory have shown that

when the oxygen is lowered rapidly, the subject compensates to a lower percentage than is possible when the rate of decrease in the oxygen is slower. The following cases illustrate the point. G. F. H. in a rebreathing experiment of 24 minutes compensated to 6.3 oxygen, but in one of 85 minutes he reached only 8.5 per cent. F. D. in 36 minutes compensated to 7.3 per cent, and in 90 minutes to 8 per cent oxygen. Unfortunately the low pressure chamber experiments for each were terminated for other reasons than failure of physiological compensations.

The data obtained from seven comparative experiments with the Dreyer nitrogen dilution method of giving low oxygen and the rebreathing method are given in table 2. Unfortunately in this series the attempt was not made to reproduce exactly in rate and low percentage the conditions of low oxygen experienced in rebreathing by the subject. The two examinations were never made on a man during the same day. The air breathed was under normal atmospheric pressure. In both we deal with a gradual decrease in oxygen percentage, that is to say, partial pressure of oxygen. The results show the similarity that was to be expected. The total acceleration and the plotted curves of the gradual increase in pulse rate corresponded in all of the comparisons made. The comparative sets of experiments on R. S. S., J. B. H., L. S. L. and P. S. B. show a very satisfactory parallelism. The onset of the acceleration was delayed longer in several of the experiments of this series than in any of the low pressure chamber series. P. S. B. in both the Dreyer and the rebreathing method showed no heart rate response until 11.3 per cent oxygen was reached, approximately 16,000 feet.

Changes in the heart rate while a low oxygen level is maintained. The majority of these experiments were conducted in the low pressure chamber. The barometric pressure was lowered to 425, 395 or 380 mm. Hg. (15,000, 17,000 and 18,000 feet) at the rate of 1000 feet per minute and held at that pressure for periods varying from 30 to 130 minutes, the pulse rate being taken every minute during the entire period. We selected these pressures because most men would stand them without discomfort or noticeable loss in efficiency.

In fifty cases the average pulse rate at 760 mm. was 74 per minute which points to a lack of anxiety in the subjects. The maximum rate for the men taken to 425 mm. (15,000 feet) was 89, and for 40 men at 395 and 380 mm. it was 94. The increase in rate at 425 mm. Hg. ranged between 5 and 19 beats. The percentage of acceleration ranged between 5.8 and 30. The average percentage acceleration in rate was

TABLE 2

Comparison of the rebreathing and diluted nitrogen experiments. Oxygen tension reduced gradually until the end

NAME	DATE	REBREATHING DILUTED NITROGEN	LENGTH IN MINUTES	FINAL OXYGEN		PULSE			BEGINNING OF ACCELERATION		
				Per cent	Tension	Begin	End	Per cent increase	Minute	Per cent O_2	Tension O_2
I. M.	3/23/18	Rebr.	28.5	7.6	57.8	78	117	50.0	6	18.0	137
	5/11/18	Dil. N.	30.0	6.0	45.6	78	110	41.0	20	11.3	86
R. S. S.	5/21/18	Dil. N.	29.0	9.1	69.1	72	96	33.4	At once		
	5/24/18	Rebr.	29.3	9.1	69.1	70	94	34.3	4	19.5	148
J .B. H.	5/17/18	Rebr.	28.0	7.8	59.2	78	93	19.2	15	13.6	103
	5/21/18	Dil. N.	29.0	7.6	57.8	72	87	20.8	22	11.0	84
L. S. L.	3/18/18	Rebr.	25.0	8.8	66.9	68	94	37.3	4	19.0	144
	5/21/18	Dil. N.	28.0	8.6	66.2	69	84	40.6	22	13.5	103
C. F. W.	5/ 6/18	Rebr.	28.0	6.8	51.6	88	116	31.8	14	13.8	105
	5/13/18	Dil. N.	32.0	5.5	41.8	76	104	36.8	16	13.4	102
A. L. D.	5/25/18	Dil. N.	28.0	6.6	50.2	86	114	32.5	14	14.5	110
	5/24/18	Rebr.	26.5	9.3	70.7	88	128	45.5	*		
P. S .B.	5/ 6/18	Rebr.	28.0	7.7	58.5	80	100	20.0	20	11.3	86
		Dil. N.	30.5	6.5	49.4	78	102	23.7	21	11.3	86

*Obscured by psychic rise.

18.7. The increase in rate at 380 mm. (18,000 feet) ranged between 6 and 45 beats. The percentage augmentation varied between 6.7 and 59, with an average increase in rate of 26.3 per cent above the normal at 760 mm.

The maximum pulse rate usually did not occur simultaneously with the arrival of the desired altitude. The lag was somewhat longer in the men taken to 380 mm., than in those at the lower altitude. In two cases in which the pressure was only 425 mm., the maximum rate was attained during the ascent. In the other men held at this level the lag varied between 2 and 7 minutes. The average lag at this pressure

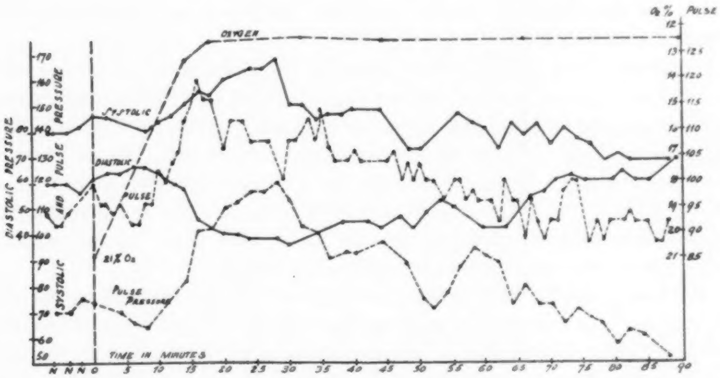

Fig. 2. K. D. Taken by the rebreathing method to 12.5 per cent oxygen (13800 feet) in 18 minutes and maintained at that level. Blood pressures and pulse rate taken every minute. This chart illustrates the return of the circulatory factors, while the low oxygen is being maintained, toward their original values. See table 5.

was 2.8 minutes. In all of the experiments in which a pressure of 380 mm. was reached and then maintained the average delay in the appearance of the maximum pulse rate was 6.6 per minutes. Every man showed some lag. In two the delay was only for 1 minute, but in several it was as long as 20 minutes, and in one the heart rate continued to increase gradually for 26 minutes, accelerating during this period from a rate of 90 to one of 108 beats per minute.

We also carried nine men by the Dreyer method to 10 per cent oxygen (19,400 feet) in 20 minutes and then held them at that level. In these cases a definite lag in the pulse rate in reaching its maximum

was observed. The shortest time taken to reach the maximum after the oxygen level was established was 3 minutes, the longest 38 minutes. The average lag was 14 minutes. These results compared with those obtained in the low pressure chamber seem to indicate that the lower the partial pressure in the air respired the slower will be the pulse in reaching its maximum rate.

Observations on the development of cyanosis are suggestive of the fact that the available oxygen is gradually decreasing within the blood during this period in pulse lag. Some cyanosis has been observed during the holding period for each level studied, but it was most conspicuous in the low pressure chamber at 380 mm. Hg. (18,000 feet). The cyanosis comes on slowly and, like the pulse rate, requires some minutes to reach its greatest degree.

In tables 3 and 4 have been tabulated the circulatory data obtained in forty experiments in the low pressure chamber in which the pressures were gradually reduced and then maintained at some time at 425, 395 and 380 mm. Hg. These data show that in many men the pulse rate does not maintain its maximum during the holding period at low pressure. Corbett and Bazett (13) on subjecting men to a constant per cent of low oxygen, observed for the pulse rate that "as adaptation takes place it tends to fall to a slightly lower level."

We find three types of pulse rate reaction during the holding period. These are: *a*, a definite and gradual decrease in rate after a brief period of maintained maximum; *b*, maintenance of the maximum rate; and *c*, a steady but slow rise in rate throughout the entire holding period. Our forty are distributed as follows: Type *a*, 29; *b*, 9; and *c*, 2. The amount of fall that occurs with adaptation is an individual matter. In some it is slight, only two to four beats, but in others the rate may return very nearly to normal. Other methods of subjecting to low oxygen gave similar results. During the holding period at 10 per cent oxygen with the Dreyer method nine men (see table 5) reacted as follows: five had a gradual retardation after maintaining the maximum pulse rate for a short time, three maintained the maximum rate and one gave a steady rise to the end.

In one long experiment with the rebreathing apparatus after the oxygen had been reduced to 13 per cent (12,400 feet) in 18 minutes, this level was maintained for 60 minutes by admitting pure oxygen into the reservoir. The pulse rate accelerated to 115 beats per minute from a normal of 92 when the low level of oxygen was reached. The rate retarded gradually to 92 during the following 80 minutes. We shall

TABLE 3

Experiments in the low pressure chamber. Subjects taken to 18,000 feet (380 mm.) in 18 minutes and maintained at that level

NAMES	DATE	PULSE									SYSTOLIC									DIASTOLIC								
		0	5	10	15	25	35	55	75	95	0	5	10	15	25	35	55	75	95	0	5	10	15	25	35	55	75	95
N. E. F.	6/ 7/18	73	75	75	79	82	79	76	72		114	112			100	102	104	100		70	72			56	58	58	54	
R. S. S.	6/10/18	70	74	76	80	94	90	95	88		118			114	114	110	104	100		70			64	54	48	42	42	
M. G. B.	6/11/18	72	74	78	82	90	88	86	82	97	118				112	112	110	112	112	70				66	68	62	64	
W. B. M. 1	6/12/18	62	62	62	68	78	78	78			118				118	118	112	118		82				72	58	56	54	
A. W. L.	6/18/18	93	93	96	98	98	100	101	101		118	120	122	124	120	116	116	118		76				78	64	56	60	
B. M. L.	6/17/18	68	69	70	78	89	90	87	85		102				102	102	102	102		68				52	48	46	46	
G. C. W.	6/25/18	60	62	64	70	78	78	78	82		100				120	120	118	114		60				64	58	48	48	
E. W. B.	6/30/18	75	80	81	84	88	86	82	84		120				144	134	130	150	140	74				74	62	64	78	70
J. M.	7/ 1/18	75	78	87	90	98	86	86	82																			
B. F.	7/ 2/18	78	78	84	100	123	114	106			130	126	124	124	136	130				70				60	52			
D. T. R.	7/ 3/18	69	72	72	76	87	87	90	88		114	112	112	114	118	116	116	114		80							78	
H. J. M.	7/ 5/18	80	83	89	93	100	97	92			108					112	114			64					48	48		
A. F. H.	7/ 8/18	72	72	75	87	88	87	84	84		104						94			70						66		
P. S. B.	7/ 9/18	75	78	79	81	89	89	87	82		110				118	118	112	112		90				64	68	70	78	
W. H. G.	7/10/18	78	82	90	97	100	98	96	93	94	110	110	108	114	120	116	116	114	110	70				68	68	70	64	64
E. A. R.	7/13/18	72	72	70	88	90	92	88	86		120	120	118	118	124	118	116	116		72				76	72	68	72	
A. F. H. 2	7/15/18	69	69	72	72	78	81	79	72		106	100	98	98	100	104	102	108		70				70			72	
W. C. W.	7/16/18	66	69	72	75	88	86	82	90		104		106	104	102	104	104	102		70				52	44	64	60	
L. G. R.	7/19/18	90	93	93	93	96	92	95	92		114			100		102	100	100		82			78		78	76	74	
L. J. S.	7/26/18	65	70	73	78	95	95	93			110				104	104				70				70	74			
A. F. H. 3	7/30/18	78	81	81	86	86	87	90			112		112	108	108	108	96			68		62	70	72	62	58		
W. B. M. 2	7/31/18	60	62	62	68	82	76	80			126				114	114				68				66	60			
B. R. L.	8/ 5/18	84	85	85	88	90	86	84	82	83	104	102	100	102			92	98	102	62			62			60	66	64
W. B. M. 3	12/27/18	69	69	78	90	110	100	100	96																			
E. C. S.	4/29/18	76	76	80	84	98	100	108	105		108					128	134		126	62					62	58		58
K. O. N.	4/30/19	84	84	89	94	102	98	95	92		116				122	120	116	116		68				64	58	56	54	
N. E. B.	5/ 6/19	74	74	76	79	86	85	85	84		108					112	106	104		76					70	70	66	

TABLE 4

Experiments in the low pressure chamber. Subjects taken to 15,000 feet (428 mm.) in 15 minutes and maintained at that level

NAME	DATE	PULSE									SYSTOLIC									DIASTOLIC								
		0	5	10	15	25	35	55	75	95	0	5	10	15	25	35	55	75	95	0	5	10	15	25	35	55	75	95
W. H. G.	5/24/18	76	82	85	94	93	91	90			100	102	102	102	104	104	100	100		66			60	62	66	68		
W. B. M.	5/27/18	63	66	68	76	82	79	80	76		110	118	118	110	110	108	110	112		76	78	78	76	76	74	74	74	
W. O. K.	6/ 6/18	76	76	77	82	89	89	85	88		120	120	116	118	128	114	116	124		68					66	66	68	
F. C. P.	12/ 9/18	72	72	74	78	80	77	75	74																			
T. S. G.	12/10/18	70	76	80	83	82	76	75																				
C. P. C.	12/13/18	84	88	94	98	95	92	88																				
T. S. G.	1/ 7/19	68	70	72	73	74	72	74	75	75																		
A. F. H.	1/ 9/19	84	90	94	103	105	105				Taken to 16,000 feet in 16 minutes																	
The following taken to 17,000 feet (395 mm.)																												
V. D.	6/26/18	86	92	98	99	99	97	94			118	116		128	116	114	108			74	76		84	80	78	84		
J. H. N.	12/31/18	74	70	78	80	88	85	80	76																			
F. D.	1/13/19	72	75	81	99	90	85	78	78																			
W. O. K.	1/14/19	63	64	70	84	90	88	84	80	80																		
K. O. W.	1/16/19	75	78	78	87	96	93	90	85																			

attempt to account for this falling off in rate during the holding period in a later paper.

The striking similarity of the pulse rate responses to a change in barometric pressure and to a decrease in oxygen percentage under normal atmospheric pressure is shown in our experiments with a gradual decrease in oxygen tension, and in the experiments in which a constant low oxygen tension was maintained. The changes in the partial pressure of oxygen give the only adequate explanation. Decreased barometric pressure and lowered oxygen percentage at 760 mm. Hg. result in the same effect on the partial pressure of oxygen, both that of the respired air and also of the alveolar air. Therefore no difference in bodily response to the two methods of producing low oxygen tension should be expected. The low pressure chamber and rebreathing experiments show definitely that barometric pressure *per se* is not a factor.

It has been observed in a number of our experiments in the low pressure chamber that, when the pulse rate falls very definitely, the color of the subject improves. Improvement of cyanosis is suggestive of better oxygenation of the blood.

Changes in arterial pressures during a gradual reduction of the oxygen tension. The blood pressures during exposure to the low oxygen of rebreathing have been described by Schneider (12). He reports three types of circulatory reaction to this form of oxygen want.

The first, the optimum, in which the pulse rate accelerates moderately as the oxygen decreases, the systolic pressure is unchanged or shows a terminal rise of not more than from 20 to 30 mm. Hg., and the diastolic pressure remains unchanged or rises slightly. The second, the controlled diastolic fall, in which the pulse rate accelerates moderately and the systolic pressure rises as the diastolic pressure gradually falls. The third, the fainting type, in which after a period of fair, good or excessive response in the rate of heart beat to low oxygen the diastolic pressure suddenly falls and soon thereafter the systolic pressure, and the pulse rate slows.

The optimum type was found to be able to tolerate as low oxygen as 6 per cent, and to lose consciousness without fainting. The fainting type rarely endured as low an oxygen percentage. The pulse pressure was found to remain fairly constant until the oxygen had fallen to between 12 and 9 per cent, after which it increased in amount during the further reduction of oxygen.

In our comparative experiments, by means of low barometric pressures in the low pressure chamber and with low oxygen percentage by the rebreathing and Dreyer methods in which the decrease was grad-

ually carried on until the subject became inefficient, we obtained by each method examples of the types of circulatory reaction described above. The parallelism in the arterial pressures is shown in tables 1 and 2 and figure 1. The parallelism in arterial pressures also emphasizes the fact that barometric pressure in itself is not the causative factor.

Changes in arterial pressure during an exposure to a constant low oxygen tension. Corbett and Bazett (13) studied aviators by means of the Dreyer method and found during a period of low oxygen tension, as a general rule, "the pulse pressure increased by a lowering of the diastolic pressure." The systolic pressure showed a slight rise. In cases in which the pulse pressure rise was due to an increase in the systolic pressure, they attributed it to mental or physical work. They believe that the

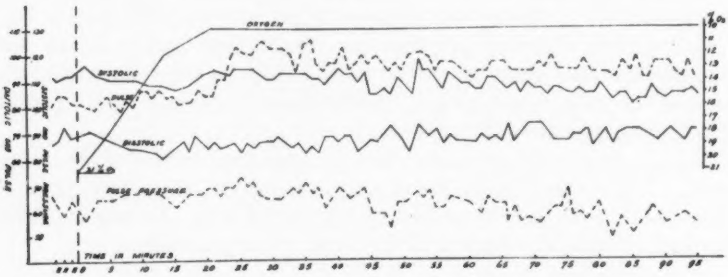

Fig. 3. W. A. B. Taken by the nitrogen dilution method to 10 per cent oxygen (19,400 feet) in 22 minutes and maintained at that level. The pulse pressure gradually decreased after the low oxygen level was reached due to convergence of the blood pressures. The diastolic pressure was little changed during the holding period. See table 5.

diastolic pressure never falls below a level of 60 mm. Hg. in a good type of reaction.

We have tabulated our arterial pressure data for the low pressure chamber and other low oxygen experiments, in which a constant level was maintained, in tables 3, 4 and 5. Since the arterial pressure variations seem to be the same for the several methods of experimentation, they may be discussed collectively.

The systolic pressures run certain clearly defined courses which may be grouped in three classes. The majority of men maintained their normal systolic pressure throughout the entire exposure to low oxygen. A few of these showed a fall at the end of the test and this was associated with oncoming syncope. In approximately 25 per cent of all

cases there occurred a slight rise in the systolic pressure during the middle of the holding period, and then during the last part of the experiment a gradual but slight fall in the pressure took place. A third group showed a gradual fall in the systolic pressure after the low oxygen level was reached. In some of these cases the fall, although slight, from 118 to 100 mm. in one, was progressive throughout the test. In others the fall appeared during the mid-period, after which the pressure held on a level until the close of the experiment.

The variety of systolic pressure changes during short exposure to low oxygen calls to mind the early observations on systolic blood pressure made at high altitudes. There was first a lack of agreement; some found an increase, others no change, and still others a fall in the systolic pressure during the sojourn at a high altitude. Schneider and Sisco (8) working on Pike's Peak concluded that in the majority of healthy men the arterial pressures are unchanged at high altitudes but that some men experience a moderate decrease in the systolic and pulse pressures, the diastolic pressure remaining most constant. The variety of systolic pressure changes is to be expected when one considers that the systolic pressure is to a large extent the resultant of other circulatory factors and compensations. The types of circulatory response are undoubtedly influenced by the severity of the low oxygen exposure with respect to the individual. Just as a man may show a different type of response at 22,000 feet from that at 15,000 feet so may two individuals with unequal compensatory ability show different types of response at a given altitude. Some men tolerate 18,000 or 20,000 feet for half an hour with little disturbance except increased heart rate. Others may have a circulatory collapse before 20,000 feet is reached.

The diastolic pressure changes observed during exposure to a constant low oxygen tension also show some variety. It should be borne in mind that during the period when the reduction in oxygen is being made, the diastolic pressure usually is not affected unless the oxygen is very much reduced, 9.5 per cent or less (21,000 feet). In a certain proportion of cases, however, at the tensions of oxygen corresponding to an altitude of 15,000 and 16,000 feet a slight lowering of this pressure may occur.

In our low pressure experiments we have only occasionally had a subject that showed a decrease in diastolic pressure during the period in which the barometric pressure was being lowered to 380 mm. In three out of some twenty cases in which, under normal atmospheric pressure, the oxygen was lowered to 12 and 10 per cent, a slight fall in pressure occurred during the ascent.

During the period of maintained level the diastolic pressure may remain constant, or it may fall slowly to a new level and hold there. Frequently after a subnormal level of varying duration it regains in some degree the normal level. The return may be only slight but it is sometimes complete. In some cases the diastolic pressure falls very slowly throughout the entire experiment, and in a few instances the experiment was terminated because of a very sudden and decided drop in the pressure.

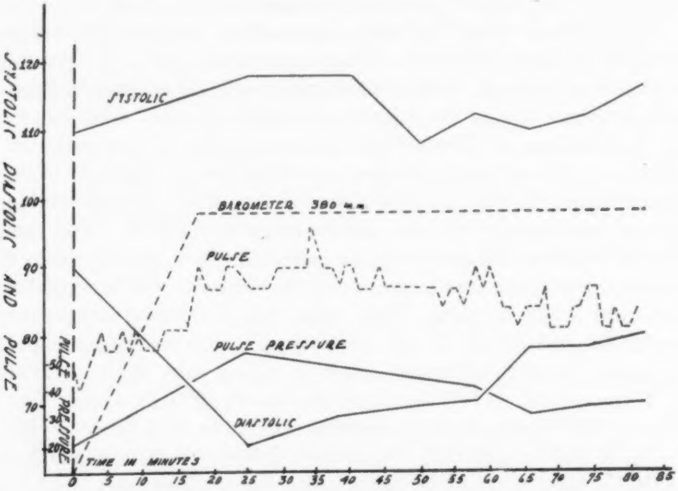

Fig. 4. P. S. B. Taken to 380 mm. (18000 feet) in 18 minutes in the low pressure chamber and maintained at that level. This case illustrates the return of the circulatory factors toward their original values. Compare with K. D. by the rebreathing method, figure 2. See table 3.

Scarcely 20 per cent of all our cases have maintained a constant diastolic pressure throughout the entire experiment. At least 70 per cent of all cases showed a fall in the diastolic pressure during the holding period. The time of its onset was variable. As pointed out, it sometimes began during the ascent, it frequently began during the first five minutes of the hold and in some instances the fall did not begin for 25 or 30 minutes. The fall in diastolic pressure takes place gradually. It requires, as a rule, 10 or more minutes to reach the level. The amount of fall in the diastolic pressure in our low barometric pres-

sure experiments ranged from 4 to 28 mm. Hg. It averaged 12 mm. at 380 mm. In the experiments with low oxygen at normal atmospheric pressure the fall in diastolic pressure varied between 6 and 26 mm. Hg.

The partial or occasional complete return of the diastolic pressure toward the normal after a period of lowered pressure was observed in at least a third of the low pressure experiments and in over 50 per cent of the experiments in which the oxygen percentage had been lowered. We believe that the return of diastolic pressure toward its normal level occurred in those cases which compensated best to low oxygen. The gradual continuous and the sudden terminal fall in diastolic pressure are associated with poor compensation.

The pulse pressure, during the holding period in both kinds of experiments, showed three rather frequent conditions which were chiefly dependent upon the course of the diastolic pressure. When the diastolic pressure was constant, the pulse pressure was, as a rule, constant. In the cases in which the diastolic pressure fell, there was a corresponding rise in the pulse pressure and when it tended to return to normal after a subnormal period, the pulse pressure fell proportionately. The decrease after the preliminary rise occurred in the majority of cases. In exceptional cases the pulse pressure either held its increase throughout or continued to increase slightly until the close of the experiment. There were five cases in which for a time the pulse pressure fell below the normal (see B. R. L. and A. F. H., table 3; V. D., table 4; W. A. B. and C. W. A., table 5). In each of these the subnormal pulse pressure period was the result of a fall in the systolic pressure.

Fainting occurred four times during the holding period at 380 mm. Hg. In these the pulse rate suddenly decreased and the character of the pulse changed. The systolic and diastolic pressures fell far below normal, the diastolic fall preceding, as a rule, the systolic. Administration of oxygen quickly restored the subject.

The above observations on the heart rate and the arterial pressures indicate that a reduction in available oxygen calls forth definite circulatory responses. The work of the circulatory system is governed by the needs of the tissues. The demand for oxygen in sufficient amounts to maintain metabolic activity remains constant during rest. When the supply of available oxygen is suddenly reduced, the tissue oxygen demand must be met by compensatory responses which will supply these needs. This burden of meeting the demand for oxygen could be taken over within limits by the circulatory mechanism. If

TABLE 5

Experiments by the diluted nitrogen method. Subjects taken to 10 per cent oxygen in 17 to 24 minutes and maintained at that level

NAME	DATE	PULSE									SYSTOLIC									DIASTOLIC								
		0	5	10	15	25	35	55	75	95	0	5	10	15	25	35	55	75	95	0	5	10	15	25	35	55	75	95
W. A. B.	5/31/18	80	81	84	84	102	99	96	93	93	112	110	110	108	114	112	110	104	104	68	66	64	62	64	64	68	68	68
I. M. M.	5/27/18	90	93	99	100	105	116				130	126	124	128	132	110				88	88	82	80	80	68			
A E C.	6/ 1/18	79	79	79	79	90	94	94	92	94	110	110	106	110	118	112	112	110	106	72	72	72	72	80	72	62	66	64
C. N.	5/29/18	58	62	66	76	84	88	84	84	84	118	118	114	118	114	110	116	118	118	76	72	74	70	66	66	54	62	50
G. B. H.	5/28/18	88	90	92	95	103	102	98	102		110	112	112	110	112	116	110	104		70	70	70	66	66	64	62	54	
E. A. R.	6/ 4/18	68	70	72	73	74	78	78	78		106	112	110	110	104	104	110	112		68	68	70	70	70	66	62	64	
C. W. A.	5/25/18	90	90	96	90	104	101	101			116	112	108	108	118	118	110			84	84	86	82	82	78	78		
R. S. S.	5/27/18	66	66	70	70	86	95	94	88		124	126	122	118	108	112	112	118		72	72	72	70	62	56	52	64	
I. M.	5/28/18	72	74	82	84	88	92	103	94	90	112	108	110	110	110	110	114	110	112	80	80	80	76	70	72	72	72	76
K. D.*	1/ 2/18	92	92	99	114	108	104	98	92		142	142	144	152	164	148	142	134		72	74	74	68	48	50	56	70	

* Rebreather to 12.5 per cent oxygen in 18 minutes and held.

the blood contains less oxygen per unit than normally, more blood must flow to the tissues to bring the required amount.

Our experiments give evidence of increased blood-flow in the acceleration of the rate of the heart beat, and in the fall of diastolic pressure with the resulting increase in pulse pressure, which occur in the majority of men held at the low oxygen level. The fall in diastolic pressure is evidence of a peripheral relaxation of the arterioles to allow more blood to pass through the tissues. The increase in the rate of heart beat with such blood pressure relationships as we find in the majority of our examinations is evidence of increased per minute output of the heart.

If this interpretation is accepted, certain features in our experiments will be found suggestive. In a considerable proportion of all men examined the pulse rate, after reaching its maximum, maintained that rate for a time and then slowly retarded. In many of these cases the diastolic fall began either before the heart reached its maximum rate, or when it arrived there. Then followed a period during which the pulse pressure continued to increase while the heart maintained its high rate. It soon took a constant level which was maintained for a period of considerable length. Still later the heart rate slowly retarded and the pulse pressure gradually fell. This would indicate that a marked and progressive increase in the rate of blood flow occurred during the reduction and early holding periods, which was followed by a period of more or less constant rate of flow. Later, as evidenced by the heart retardation and rise in diastolic pressure, the flow of blood returned somewhat toward the normal rate. The validity of this interpretation may be questioned because a retardation in the blood flow occurs when the needs of the tissues for oxygen remain unaltered. The observations on cyanosis should here be borne in mind. At 380 mm. Hg. pressure, some subjects were for a time very cyanotic and later, when the heart rate was retarding and the diastolic pressure rising, the color improved. Some subjects felt wretched during the first part of the holding period only to report later that they were feeling much better. We believe that the retardation of the heart rate, the return of diastolic pressure toward normal, the decrease in pulse pressure and the improvement in color and feeling occur when other compensatory mechanisms have become effective. We plan to report observations concerning the problem in another paper.

SUMMARY

1. Low oxygen tension effects were studied during a period of gradual decrease and while a level was maintained for from 30 to 130 minutes. Three methods were employed to obtain low oxygen tensions, the low pressure chamber, rebreathing of air at normal atmospheric pressure, and air diluted with nitrogen.

2. The effects of low oxygen tensions upon the circulatory mechanism are the same regardless of the method used to vary the oxgyen. Barometric pressure *per se* is not the causative factor.

3. The heart rate responds to slight changes in oxygen tension. The acceleration in the majority of men examined began between oxygen partial pressures of 113 and 128 mm. corresponding to barometric pressures of 542 and 610 mm. (5800 and 8800 feet). In at least 25 per cent of all cases the first response occurred at oxygen partial pressures of about 137 mm. or less corresponding to a barometric pressure of 656 mm. (4000 feet). The initial response occurred at about the same oxygen tension each time a subject was exposed to a decreasing oxygen tension by the several methods used.

4. The increase in rate of heart beat differed in individuals, but was found to be the same for an individual when the rates of decreasing tension and the partial pressures reached were the same in the comparative experiments.

5. When a constant level of oxygen was maintained, the heart reached a maximum rate after the lapse of a period of variable length. This maximum was maintained for some time in the majority of men, after which the rate returned in greater or less degree toward the normal rate. In others the maximum rate once attained was continued to the close of the experiment. A few men showed a gradual increase in rate throughout the whole period of the constant low oxygen level.

6. The systolic pressure maintained its normal level in the majority of cases. In 25 per cent of the cases a slight rise occurred during the first part of the experiment, falling gradually as the experiment proceeded. In others a gradual fall began soon after the desired altitude was attained.

7. The diastolic pressure usually fell gradually about 4 to 28 mm. during a period of a maintained oxygen tension. In many of the experiments it later returned somewhat toward the normal. In some cases the diastolic pressure continued to fall gradually throughout the entire experiment. In a few men the pressure was unaltered by the low oxygen.

8. The pulse pressure usually increased during the time of the holding period at a given oxygen tension. It ordinarily followed the diastolic pressure changes in an inverse way.

9. The bearing of the pulse rate and blood pressure changes on blood flow are briefly discussed.

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XVIII. CONDUCTION IN THE SMALL INTESTINE

WALTER C. ALVAREZ AND ESTHER STARKWEATHER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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During the last year we have brought forward considerable evidence in favor of the view that there is a metabolic gradient in the intestinal wall from duodenum to ileum (1). Per unit of weight and time the duodenal muscle gives off more CO_2 than does the ileal muscle. That this is not due simply to its greater activity is shown by the fact that the difference can still be shown when both segments are kept paralyzed by adrenalin. It is our belief that this metabolic gradient underlies and gives rise to gradients of rhythmicity, latent period and irritability which determine the direction of the diastaltic waves.

It next occurred to us that such a metabolic gradient might influence the conduction of stimuli up and down the intestine. We should expect such stimuli to travel farther and faster with the gradient than against it. It seemed worth while to look into the matter not only because of its academic interest but because the demonstration of such differences in conduction might throw light upon the mechanism of the "myenteric reflex" and upon various clinical problems. When we contemplate the tremendous advances which have been made in medical knowledge through the study of conduction in the heart muscle we must the more lament our almost complete ignorance as regards conduction in the intestine.

Schillbach (2) thought the contractions resulting from electrical stimulation of the bowel ran farther upwards than downwards. Bayliss and Starling (3) found it hard to show descending inhibition in the rabbit because it extended at most 2 to 3 cm. and was so fleeting that it did not alter the appearance of the tracing to any great extent. In no case in the rabbit did excitation spread more than 5 to 6 cm. upwards. They had difficulty in showing any spread of the stimulus in the cat's bowel unless they gave castor oil. In the dog, the descending impulses traveled farther than the ascending. In fact, they could

not show the ascending excitation unless they stimulated just aborally to the recording balloon. On the other hand, they sometimes obtained effects two or three feet below the point stimulated (4). Conduction was slow—about 10 cm. per second. They had difficulty in estimating this rate on account of the long and variable latent period. After painting the bowel with cocain or after injecting the animal with nicotin the waves seemed to run equally well in either direction at a rate of 2.3 cm. per second. Stimuli evoked little response in these poisoned bowels.

There are a number of observations in other fields which would lead us to expect a better conduction in the aboral than in the oral direction. Child (5) has shown in many tissues that there is a constant stream of inhibiting influences descending along the gradients of metabolism which he has demonstrated. Tashiro (6) has explained the polarity of nerves on the same basis. The gradient of CO_2 production descends peripherally in motor nerves and descends centrally in sensory nerves. MacArthur and Jones (7) have shown a gradient of oxygen consumption in the central nervous system descending from the brain to the end of the cord. When angle worms (8), planarians and centipedes (9) are cut in two, the forward end may crawl on undisturbed while the hind end writhes convulsively. One explanation of this phenomenon is that conduction is almost entirely in the aboral direction. Carlson (9) has shown in the myriapoda that conduction along the ventral nerve cord is more rapid in the aboral than in the oral direction. This is true also for the spinal cord of the hag-fish (10) and probably for the cord of the snake (11). Of course in the spinal cord this difference might perhaps be due to a greater length and simplicity of the downward conducting paths. Sherrington has shown with the spinal cord of higher animals that inhibition spreads downward more easily than upward. In the decerebrate cat it is much easier to obtain reflexes in the hind limbs by touching the head than to get movements of the head or fore-limbs after touching the tail. "The exclusively aboral direction taken by shock seems to be universal in the nervous system" (12). It is suggestive that in the cord the region just above an injury seems often to be in a stimulated and hyperesthetic state much as the corresponding segment should be in the bowel.

The simplest nerve nets such as those in the medusae and sea-urchins conduct stimuli equally well in all directions (13). Others, however, like those in the feet of snails are "polarized" so that they conduct best in one direction (14). Parker (15) has suggested that the direc-

tion of peristalsis may be due to such a polarization of Auerbach's plexus.

Technic. Most of the work has been done on the small intestine of the rabbit because its contractions are so even and regular that abrupt changes in the record can with considerable certainty be interpreted as the effects of the stimuli used. Many experiments have been done also with the bowel of the cat. The rat's intestine was so insensitive to stimuli that we made only a few attempts to use it.

In order to simplify the problem we first studied the conduction in excised loops of bowel contracting rhythmically in warm aerated Locke's solution. Such a loop is fastened to the bottom of the vessel by serrefines which grasp it at two points 3 cm. distant from the ends. These short end segments are then turned up like the vertical arms of a U and connected to heart levers by means of threads. By turning up the recording ends of the loop in this way, one can avoid the use of pulleys.

In order to get a longer stretch of bowel between the recording ends and to have the bowel more accessible for the various types of stimulation, we replaced the usual tall narrow beaker with a long, comparatively shallow, glass bread-baking dish. On the bottom of this dish was a strip of cigar-box wood held down with lead weights. A centimeter scale was marked on its upper surface and at one end of the scale was fastened a little wire serrefine. At the other end was nailed a wooden upright with a little ring at its base and a cleat at the top. The segments were fastened in this apparatus as shown in figure 1. The water-bath surrounding the glass dish was kept at 38°C.

In some experiments the bowel was stimulated by pinching or by applying a crystal of NaCl. The most convenient form of stimulation and the only measurable one was the faradic shock. A convenient electrode for underwater work was made by inserting the wires from the secondary coil into two L tubes the lower ends of which were fastened on a short bar so that the bowel would just fill the space left between them. (See fig. 1.) A few experiments showed that the current kept very closely to the short path between the two tubes. A Harvard induction coil was used with one dry cell supplying 20 amperes at 1.7 volts. A tetanizing current was used.

In most of the tracings the movements recorded are those of the longitudinal muscle. When the serrefines were placed so that the circular muscle was recording, the amplitude of contraction naturally was less; the tendency to beat rhythmically was less and the irritability

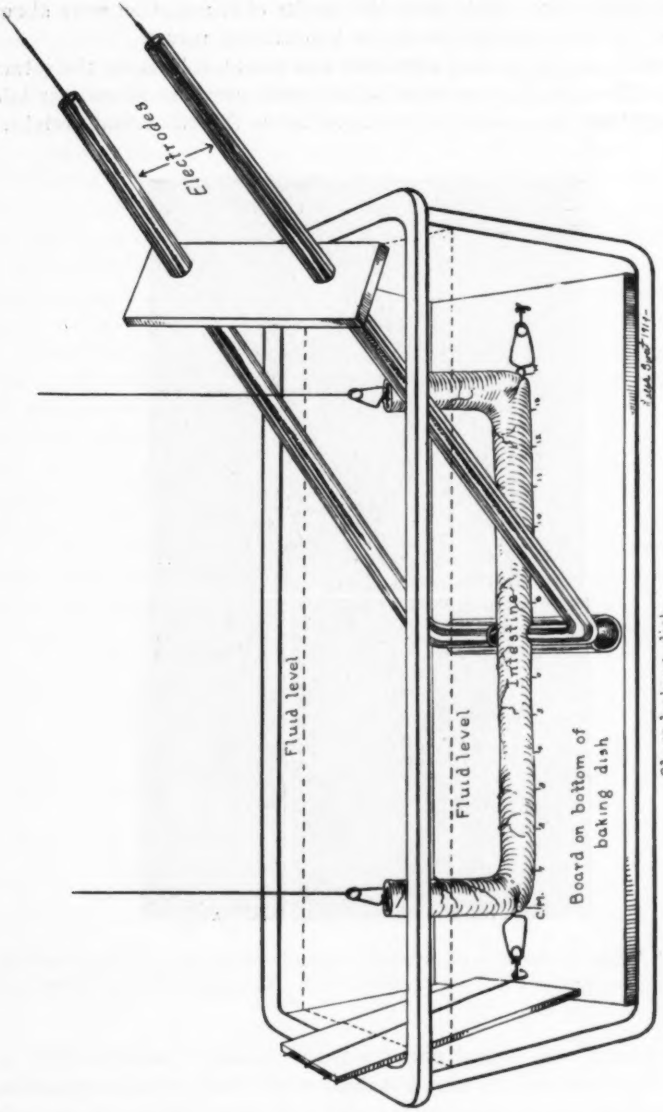

Fig. 1. Apparatus used in studying excised segments. For details see the text.

was often very low. Otherwise the results of stimulation were about the same as those obtained with the longitudinal muscle.

The work on the excised segments was repeated later on the intact bowel. The animals were anesthetized with urethane (2 gm. per kilo by mouth) and the cord was destroyed below the 4th dorsal vertebra.

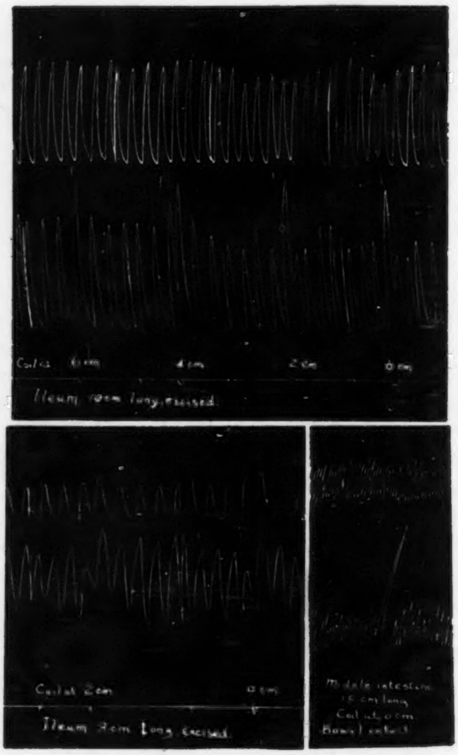

Fig. 2. When the bowel is stimulated midway between the recording segments the effects are most marked on the aboral end. The upper tracing is from the orad end.

The abdomen was opened under a bath of Locke's solution kept at 38°C. The best records were obtained with a very simple apparatus. Two glass rods were held vertically with their lower ends in the animal's abdomen. Fastened to the ends were little serrefines which seized the bowel at two points from 5 to 50 cm. apart, and held it down. Other

serrefines, attached by threads to the levers, were so arranged that two segments, 3 cm. long, and the desired distance apart, recorded their contractions on the drum. In some experiments we used the enterograph designed by Alvarez (16); in others we used balloons.

Conduction better in the aboral direction. The conclusions reached in this paper are based upon an analysis of over 2200 reactions. There were about 1000 each on the excised and intact intestines; 1700 were the results of electric stimuli; 400 the results of pinches and cuts, and the rest were the results of stimulation by salt crystals and balloons. In practically every instance it was easy to show that conduction is better in the caudal direction. If the distance between recorders was small enough so that a stimulus in the middle affected both tracings, the disturbance was more marked in the lower one than in the upper. (See fig. 2.) With a longer distance between the two, the lower recording segment would respond well to stimulation at the base of the upper when the upper failed to respond to stimulation at the base of the lower. (See fig. 3.) In other experiments the stimulating electrodes were brought closer and closer to the recording segment until it showed some response. The following table shows some of the results obtained in this way with strong faradic stimuli. It will be noticed that in one case a response was obtained 57 cm. below the point stimulated and only 7 cm. above.

DUODENUM		JEJUNUM		MIDDLE		ILEUM	
Orad	Caudad	Orad	Caudad	Orad	Caudad	Orad	Caudad
			Excised segments				
5.5	12.5	5.5	15.0				
6.0	12.5	8.0	20.0	6.0	12.5	6.0	12.5
		6.0	15.0	6.0	15.0	6.0	13.0
10.0	18.0	6.0	15.0	6.0	15.0		
7.0	12.+	8.0	12.+	7.0	12.+		
			Intact animal				
2.5	19.5			2.5	14.5	2.5	10.0
		9.5	19.5	9.5	48.0	7.0	14.5
		5.0	19.0	5.0	19.0	5.0	20.0
				4.5	36.0	4.5	36.0
		5.0	20.0			5.0	24.0
				5.0	43.0		
		9.5	43.0	10.0	43.0	12.0	72.0
				7.0	57.0		

The figures on any horizontal line are from one rabbit.

These differences were observed also with the mechanical and chemical stimuli, but they were not so striking. The experiments in which only the hind end of a worm reacts to a cut can sometimes be duplicated with a short U-loop of excised bowel.

The myenteric reflex. It will be noticed in the tracings that the characteristic response not only at the point stimulated but in the regions proximal and distal to it is an increase in amplitude and tone followed perhaps by a drop in amplitude and tone. In all this work we have seen very few examples of what might be called a "myenteric reflex." Thinking that perhaps this was due to the fact that we were using the longitudinal muscle when previous workers had used the

Fig. 3. When the bowel is stimulated above it responds below; when stimulated below it often fails to respond above. The upper tracing is from the orad segment. The distance between the recording segments was 5 cm.

circular we repeated the work attaching the serrefines so they would pull at right angles to the long axis of the bowel. In some experiments also we used balloons as others have done. With the excised segments the circular coat was slow to start beating and was often very unresponsive to stimuli. Otherwise the results were usually the same as those obtained with the longitudinal muscle. The greatest number of typical myenteric reflexes were obtained while using balloons—suddenly inflated and deflated—as the stimulus. We were not entirely satisfied, however, with the results obtained with these balloons. The trauma attendant upon their insertion and the violent

efforts of the bowel to force them out make conditions far from normal. It may easily be that the "myenteric reflex" is primarily a response to stretching and not a response to other forms of stimulation of the gut.

Observations on different animals. The rabbit's colon was too insensitive for satisfactory work. We had the same trouble with the excised small bowel of dogs and white rats. Considerable work was done with cats. The excised small intestine beat irregularly and was often insensitive. We were able to show, however, the same peculiarities of conduction that were seen in the rabbit. One cat was decerebrated so that we could avoid the use of urethane. As our results with this animal were the same as with others, the anesthetic probably had no appreciable effect on the bowel.

Remarkable differences were found in the irritability and conductivity in different animals of the same species. Previous work makes us feel that the degree of infection with intestinal and other parasites must have a good deal to do with these differences. Often the bowel was very insensitive in spite of a good rhythmicity.

Both with the excised segments and intact animals conduction seemed better in the middle region of the small intestine than at the ends. Poor results in the duodenum and upper jejunum could easily be explained, however, by the greater reaction to trauma and handling in that region. Sometimes the duodenum would respond well only to the first stimulus.

Rhythmicity. We had occasion to confirm the previous observation of Alvarez (17) that the rate of the orad end of an excised segment of rabbit's intestine is higher than the rate of the lower end. Keith (18) has suggested, on the basis of his anatomical studies and some of Alvarez's observations, that there are a number of rhythmic centers in the bowel, containing nodal tissue and dominating the rhythm of the adjacent regions. We have not been able to show any such dominance anywhere except occasionally in the terminal ileum (17). When the aboral recording end of a segment was severed from the rest of that segment its rate of contraction was never altered to any extent. There seem to be no such descending influences affecting the rhythm as there are in the heart. It is an interesting point, however, that when the longer segments were put into the aerated Locke's solution the lower end usually started contracting some time before the upper. This was due probably to a greater reaction to trauma caudad to the upper cut end than orad to the lower cut end.

Rate of conduction. Theoretically, an impulse should not only travel

farther with the gradient but it should travel faster with it than against it. We have made many attempts to show this but so far have not been able to obtain trustworthy records. The normal bowel is constantly in motion so that no satisfactory base line can be maintained. The muscle is slow in its reactions so that it is hard to say just when it begins to shorten. When it is contracting rhythmically a stimulus may show itself only as an increase in the height of the succeeding wave. Sometimes there is a temporary inhibition which delays the appearance of the reinforced wave. Still stronger inhibition may show itself as a drop in tone before the rise. But even when a fairly sharp take-off can be marked on the tracing the variations in latent period are so large that they may cover up differences due to the conduction time. Moreover, the latent period at a distance from the point stimulated may be lengthened considerably because the stimulus has become weakened during conduction. The different types of response to stimuli are shown in figure 4. A good deal of work was done with segments in which the rhythmic contractions had been more or less stilled by strychnin, adrenalin, nicotin, cocain and digitalis. Segments were studied also in baths of physiologic NaCl solution where they did not beat. Although some fairly satisfactory records were obtained in this way, slight tonus changes generally persisted even when the seg nents were so badly poisoned that their irritability and conductivity were practically gone. The most conclusive data were obtained from records of actively contracting untreated segments, stimulated half way between the two ends. Ordinarily. on account of the difference in rate at the two ends of the strip, one lever would be going up while the other was coming down, but occasionally for a few seconds the two ends would beat practically in unison. If stimuli were thrown in at those times it could often be shown that a fairly abrupt alteration in the tracing appeared first at the distal end. Following are some of the time intervals obtained. The segments were from 8 to 15 cm. long. The figures represent seconds elapsed after stimulation in the middle.

Excised segments

Upper end	2.0	1.5	4.2	4.2
Lower end	1.2	0.5	2.7	1.5

Intact animal

Upper end	0.8	1.3	1.1
Lower end	0.25	1.0	0.6

The conductivity varied markedly in the different animals. In two of the rabbits the impulse travelled 40 or 50 cm. in less than one second. Ordinarily the conduction time seemed to be much shorter. Following are some of the estimates made: 9, 15, 40, 27, 7, 10, 8, 30, 50, 20, 11,

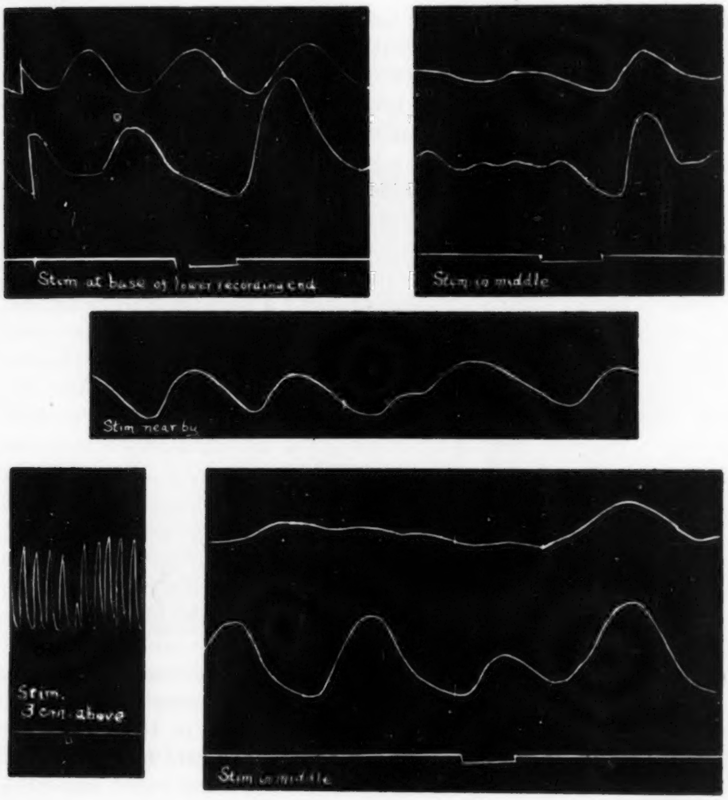

Fig. 4. To show different types of response to stimulation and the difficulties in the way of estimating the conduction time. The upper tracing is from the orad segment.

26, 16, 12, 40, 13, 22, 30, 11, 12, 40, 15. The average is 21 cm. per second. This figure would probably be higher with a more accurate technic. It represents the aboral rate Conduction orad is harder to

measure as it is ordinarily limited to short distances where the error is larger.

At first sight the slow rate suggests conduction through the muscle, but a review of the literature on nerve-net muscle combinations shows that the impulses generally travel through the net and that the rate is usually slow. Thus the rate in Cassiopea is 27 to 50 cm. per second at 25°C.; in Metridium at 21°C. it varies between 12 and 14 cm. per second (19). The nerve net not only serves to expedite conduction but it keeps the muscle from contracting down hard. Several observers have noticed that when smooth muscle is cut off from its nervous connections its tone rises to a point where rhythmic contraction is impossible (20). It may be that some of the contractions in spasmodic ileus, in infantile pyloric stenosis and in Hirschprung's disease are due not to some abnormal stimulation but to the loss of this normal inhibition.

Some evidence was obtained at times of a more rapid conduction, perhaps by way of the mesenteric nerves. On two occasions sharp reactions were observed in the lower ileum two seconds after a stimulus had been applied to the greater curvature of the stomach. The distance along the bowel was from 300 to 400 cm. Frequently marked changes in the tone and rhythmicity of the loop studied would follow almost immediately after defecation, after small peristaltic rushes, or after handling the bowel 200 cm. or more above the region observed. The tone of the whole small intestine seemed to rise during efforts at defecation and it fell suddenly when food passed through the ileo-cecal sphincter. Figure 5 shows some of these long distance reactions. Bayliss and Starling have well said that "every point of the intestine is in a state of activity which can be played upon and modified by impulses arriving at it from all portions of the gut above and below" (21).

The rush waves along the bowel in the rabbit travel about 4 cm. per second. Theoretically they should go faster in the duodenum where the gradient is steepest and the metabolic rate fastest. They certainly seem to do so in man where the resultant rapid emptying accounts for the term "jejunum." In the long bowel of the rabbit a rush wave seems often to gain headway the farther it goes so that a wave which travels 2 cm. per second in the duodenum travels 6 cm. per second in the lower ileum.

No difference in reaction with strychnin. The work of pharmacologists suggests strongly that strychnin acts mainly upon the synapses between the neurones; it makes conduction across them easier and

therefore heightens reflexes (22). It may be then that we can follow Parker's suggestion (15) and use strychnin as an index to the simplicity or complexity of the nervous system in different animals. The simpler forms of life with pure nerve nets should show only the toxic protoplasmic effects of strychnin, while the higher forms with more and more synapses should show heightened reflexes and clonic spasms. Moreover, we might find that disturbance in the mechanism of reciprocal innervation which has been observed not only in the spinal cord of mammals but even in earthworms and starfish after the administration of strychnin (23). Although considerable work must yet be

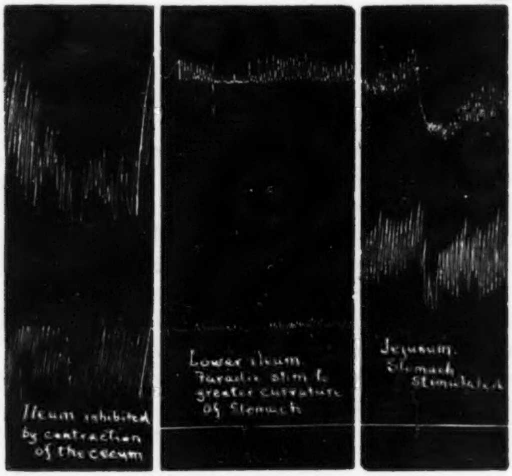

Fig. 5. Intact bowel affected by activity of the cecum and by stimulation of the greater curvature of the stomach.

done before we can say how dependable this strychnin test is, it is certainly suggestive that we have been unable to show much difference between the conduction time and the type of reaction to stimuli in strychninized and normal animals.

We kept a number of our rabbits and cats for several hours under large and repeated doses of strychnin. At no time could we show any improvement in conduction or any change in the type of reaction to stimuli, even when the animals were twitching and going into convulsions. (It should be remembered that they were under urethane

anesthesia.) Similar experiments were performed with the excised segments, beating in Locke's solution to which strychnin had been added. Frequently conduction was decidedly impaired by the drug but almost always it remained better in the caudad than in the orad direction. Auerbach's plexus then would appear to be a simple net, without reflex arcs and without synapses excepting those between the net and the central nervous system. This conclusion agrees with that of the anatomists who, after much argument and research, have finally decided that there are no commissural fibers in the involuntary nervous system such as would be necessary for the working of true reflexes (24).

SUMMARY

It has been shown that there is a metabolic gradient in the small intestine from duodenum to ileum. As would be expected, conduction is better with the gradient than against it.

The rate of conduction could not be determined accurately. It appears to be about 20 cm. per second. At times it is about 150 cm. per second, probably by way of the nerves in the mesentery.

In the rabbit, the peristaltic rushes travel about 4 cm. per second.

The characteristic response to a stimulus applied to the gut is a contraction above and below. This may be preceded or followed by an inhibitory phase. The "myenteric reflex" was rarely observed, and then usually after distension by balloons.

The tone and activity of any part of the tract can be affected markedly by the activities of other parts.

With the exception of the terminal ileum, no part of the bowel seems to affect the rate of rhythmic contraction of adjacent parts. This finding is against the theory of peristalsis offered by Keith.

The failure of strychnin to influence conduction or to alter the type of response to stimuli suggests that Auerbach's plexus is a simple nerve-net, without synapses or reflex arcs.

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